COMPARATIVE ASPECTS OF HAEMOLYTIC DISEASE OF THE NEWBORN

Comparative Aspects of HAEMOLYTIC DISEASE OF THE NEWBORN

G. FULTON ROBERTS, M.A., M.D.
Fellow of Jesus College, Cambridge and
Lecturer in Pathology in the University



LONDON
WILLIAM HEINEMANN · MEDICAL BOOKS · LTD
1957

First published 1957

By the same author
THE RHESUS FACTOR, 1947

Printed in Great Britain by
BILLING & SONS LTD.
GUILDFORD AND LONDON
H3712

CONTENTS

PREFACE

This book, though written to some extent for pleasure, is published in the hope that it may prove of interest to those who concern themselves with the care of young animals, whether in a medical or veterinary milieu. The study of haemolytic disease embraces many aspects of the physiology and pathology of the newborn animal and provokes many problems of serological and biochemical technique in the laboratory, of clinical and therapeutic assessment at the bedside, and of animal husbandry and economics in the field. But this book is no attempt at an exhaustive treatise in which all of the vast mass of writings on the topic would be mentioned and appraised. Rather, it is intended as a short account of present-day views on haemolytic disease

pretations are mine and that I have acceded to certain whims, first in attempting to express the material in the

giving a few reasons for the choice of nomenclature in a field thickly sown with near-synonyms.

The introduction is a very simple account of the disease for the assistance only of those readers who may not be familiar with its classical features.

I acknowledge with pleasure the valuable help and interest that I have received at various times from Dr R. R. A. Coombs, Dr R. F. W. Goodwin and the late Dr B. H. G. Hayward.

G. F. R.

INTRODUCTION

Haemolytic disease of the newborn results from the immunization of a mother against a blood group antigen possessed by her foetus but not by herself Blood group

immunization is to result. In some cases the means of immunization of the mother is plain to see, perhaps an incompatible blood transfusion or some other injection of blood or blood products. In other cases immunization seems to result solely from pregnancy in a way that is not at present fully understood

Once a mother is immunized against a blood group antigen of her foetus the stage is set for the appearance of haemolytic disease of the newborn. In man (as in the rabbit, in which the condition can be induced experimentally) the antibody can reach the foetus *in utero*, and exert a baneful influence on its red blood cells. In severe cases the destructive effect of the maternal antibody on the foetal cells proceeds so far *in utero* that the infant is born dead, usually in an oedematous state, a condition called *hydrops foetalis*.

disorder and may, indeed, pass unnoticed But in the majority of cases the haemolytic anaemia is severe and acute,

The introduction is a very simple account of the disease for the assistance only of those readers who may not be familiar with its classical features.

I acknowledge with pleasure the valuable help and interest that I have received at various times from Dr R. R. A. Coombs, Dr R. F. W Goodwin and the late Dr B H. G. Hayward.

G. F. R.

INTRODUCTION

Haemolytic disease of the newborn results from the immunization of a mother against a blood group antigen possessed by her foetus but not by herself. Blood group incompatibility between mother and child is quite common, but haemolytic disease does not usually ensue unless two conditions co-exist, and not always then. First, the blood [illegible]

munization of the mother is plain to see, perhaps an incompatible blood transfusion or some other injection of blood or blood products In other cases immunization seems to result solely from pregnancy in a way that is not at present fully understood.

Once a mother is immunized against a blood group antigen of her foetus the stage is set for the appearance of haemolytic disease of the newborn In man (as in the rabbit, in which the condition can be induced experimentally) the antibody can reach the foetus *in utero*, and exert a baneful influence on its red blood cells In severe cases the destructive effect of the maternal antibody on the foetal cells proceeds so far *in utero* that the infant is born dead, usually in an oedematous state, a condition called *hydrops foetalis* It is more common, however, for the process to be less far advanced *in utero*, so that the manifestations are most pronounced in the neo-natal period. In the mildest cases some degree of anaemia, which slowly mends, may be the sole disorder and may, indeed, pass unnoticed But in the majority of cases the haemolytic anaemia is severe and acute,

and is accompanied by an early development of severe jaundice, a condition called *icterus gravis neonatorum*.

In man, haemolytic disease is a cause of severe jaundice in the neo-natal period, probably associated with a general immaturity of the tissues This combination of early and pronounced jaundice with general immaturity may also be found in premature infants not suffering from haemolytic disease. Whether the combination results from haemolytic disease or from prematurity, there is a grave risk of damage to the brain, accompanied by patchy pigmentation in the central nervous system. This complication is manifest clinically in neurological disturbances which are commonly fatal, or leave permanent evidence of brain damage in those that survive This condition, called *kernicterus*, has not so far been described in any species other than man.

When maternal iso-immunization occurs in the horse or pig (or is induced experimentally in the dog) the antibodies are unable to reach the foetus *in utero*, and so in these species the disease is not initiated before birth. The young are born healthy but take the antibody, often in very high concentration, in the colostrum and milk For the first day or so of life, the ingested antibody is readily absorbed from the intestine and the animals thus receive the full force of very powerful antibody during the first forty-eight hours of life The result is an acute haemolytic anaemia accompanied in severe cases by haemoglobinuria.

In man, and in the horse and mule, the disease may apparently occur spontaneously with no evident cause of the mother's immunization except as a consequence of pregnancy In these circumstances it is common for one or more of the first-born children to escape the condition, and the disease occurs in the subsequent offspring. It seems the rule that once an infant suffers with the condition, all later siblings are affected if they carry the blood group antigen that is incompatible with the mother's antibody.

The blood groups that are responsible for iso-immunization have not been elucidated in all species In man the most important blood group antigen concerned came to be called the Rhesus factor or more briefly Rh Those possessing the antigen were called Rh or Rhesus positive, and

[illegible]

iso-immunization Unlike the Rh antigens, the A and B substances are found not only in the red cells but also in the secretions of about four-fifths of the human population, such persons are termed "secretors".

Haemolytic disease is not the only cause of jaundice in the newborn It may occur mildly in normal infants (so-called physiological jaundice) and in congenital syphilis, virus infections and other rare maladies. Nor is haemolytic disease the sole cause of oedema of the newborn which may occur in congenital disorders of the heart and kidney.

CHAPTER I

THE DISEASE IN MAN: HISTORICAL ASPECTS

Icterus Gravis Neonatorum

The Seventeenth Century

One might suppose that jaundice of newly-born infants, being both an obvious and a common manifestation, would be the subject of comment in medical writings of ancient

dition fully is that of Sylvius (1679) He attributed the condition to occlusion of the bile duct by "glutinous humours"

sistent with a diagnosis of icterus gravis neonatorum, but

no such claim could be entertained concerning an account only nineteen words long.

As early as 1673, John Otto Horst of Darmstadt offered a nine-page inaugural disputation devoted entirely to a case of an icteric childbirth. Although there are several features of thi
rather
our o
twice
days
familial neo-natal jaundice.[2] Possibly the condition was congenital syphilis, but it is not established beyond doubt
ok the
unts of
end of
estab-
lished.

The other accounts that appeared in the seventeenth century are evidently not cases of haemolytic disease of the newborn, though they are of interest in themselves[3] Three of them, those of Kerckring (1670), Schultze (1676) and Fehre (1694), concern the bearing of jaundiced infants by

other three reports, those of Anhorn (1693), Bierling (1694) and Hagendorn (1698), are of less interest, in one of Hagendorn's cases an attempt was made to wash away the jaundice.

The Eighteenth Century

The publication of these cases before 1700 enabled the authors of text-books in the eighteenth century to devote some space to the appraisal of infantile jaundice specifically, and such accounts can be read in the books of Duttel (1702),

believed to be plugged with mucus The principal interest of the paper is the description of the brain which is referred to on page 40.

The feeling that jaundice of the newborn did not excite

logical jaundice. His thesis "Traité de l'ictère des enfans de naissance" was first published in 1788, but probably in a limited edition, for it is never quoted, and seems to be inaccessible The second edition of 1806, which is more easily consulted, was more seminal, for a spate of theses on this subject, mostly in French, poured out during the ensuing years, in many of which a debt to Baumes is freely acknowledged.[5] His influence, and that of Pearson to be described, belongs to the following century.

The Nineteenth Century

It was clearly established during the early part of the nineteenth century that neo-natal jaundice might assume a mortal form, terminating often with convulsions. Full accounts of the condition appeared at this time in the textbooks of midwifery and diseases of children such as those of Gardien (1824), Burns, of the same year, and Dewees (1826) But the essential clue, which might have served to differentiate the icteric form of haemolytic disease of the newborn from other forms of severe neo-natal jaundice (except congenital syphilis), was its familial incidence. Observations on the familial nature of the condition are, however, very hard to find in the writings of this period. It seems that the first unequivocally to describe and draw attention to a series of cases of jaundice in the newborn of one family was Pearson.

The case that Pearson described is very likely to have been one of haemolytic disease of the newborn; there were nine previously affected siblings, and the clinical description is convincing Moreover, at autopsy, the bile ducts were found to be free from impediment.[6] The child described was born in 1796, but it is not clear where and when Pearson's account was published, if indeed it ever was. It is quoted, seemingly as a personal communication, in the 1799 edition of Underwood's "Treatise on the Diseases of Children", but it had not appeared in his edition of two years earlier.[7] It is also quoted verbatim, with trivial variations, in a footnote to Cheyne's second "Essay on the Diseases of Children" dated 1802; and Cheyne himself added another family, from his own observation, in which

years

Though Pearson's and Cheyne's descriptions were not referred to again until 1883, it would not be true to say

view of 1834. And among those reported as congenital occlusion of the bile-duct which are likely to have been

icterus in newborn foals, was put in mind of a neighbour of his who had lost several successive infants from jaundice

The muddle persisted throughout the century, despite several tentative observations that occlusion of the bile-duct could not be the whole story West, for instance, begins to express some doubts by the time of the fourth edition of his widely read book [10] Writing about congenital obliteration of the bile duct in 1859, he says: "death takes place sooner or later, and though now and then life is prolonged for several weeks or months . . yet in the

logical jaundice His thesis "Traité de l'ictère des enfans de naissance" was first published in 1788, but probably in a limited edition, for it is never quoted, and seems to be inaccessible. The second edition of 1806, which is more easily consulted, was more seminal, for a spate of theses on this subject, mostly in French, poured out during the ensuing years, in many of which a debt to Baumes is freely acknowledged.[5] His influence, and that of Pearson to be described, belongs to the following century.

The Nineteenth Century

It was clearly established during the early part of the nineteenth century that neo-natal jaundice might assume a mortal form, terminating often with convulsions. Full accounts of the condition appeared at this time in the text- [illegible] children such as those [illegible] ime year, and Dewees [illegible] might have served to differentiate the icteric form of haemolytic disease of the newborn from other forms of severe neo-natal jaundice (except congenital syphilis) was its familial incidence. Ob- [illegible] e, how- [illegible] riod It [illegible] d draw attention to a series of cases of jaundice in the newborn of one family was Pearson.

The case that Pearson described is very likely to have been one of haemolytic disease of the newborn, there were nine previously affected siblings, and the clinical description is convincing Moreover, at autopsy, the bile ducts were found to be free from impediment.[6] The child described was born in 1796, but it is not clear where and when Pearson's account was published, if indeed it ever was. It is quoted, seemingly as a personal communication, in the 1799 edition of Underwood's "Treatise on the Diseases of Children", but it had not appeared in his edition of two years earlier.[7] It is also quoted verbatim, with trivial variations, in a footnote to Cheyne's second "Essay on the Diseases of Children" dated 1802; and Cheyne himself added another family, from his own observation, in which

two children were affected.[8] These families, then, observed

servations lay forgotten or neglected for more than 80 years.

Though Pearson's and Cheyne's descriptions were not referred to again until 1883, it would not be true to say

view of 1834 And among those reported as congenital occlusion of the bile-duct which are likely to have been cases of icterus gravis are those of Campbell (1844), Binz *(1866), Herz (1878), Glaister (1879) and D'Espine (1880). It* seems clear that in the medical history of the nineteenth century a continuous stream of cases recognizable as haemolytic disease were published, but submerged under

icterus in newborn foals, was put in mind of a neighbour of his who had lost several successive infants from jaundice

The muddle persisted throughout the century, despite several tentative observations that occlusion of the bile-duct could not be the whole story West, for instance, begins to express some doubts by the time of the fourth edition of his widely read book.[10] Writing about congenital obliteration of the bile duct in 1859, he says· "death takes place sooner or later; and though now and then life is prolonged for several weeks or months . . . yet in the

majority of instances the fatal issue takes place within a *fortnight after birth; and this in consequence of haemorrhage from the umbilicus immediately after the separation of the funis. . . . Now and then, too, in spite of the co-existence of haemorrhage and jaundice, the former has been checked and the latter has disappeared;* the child completely recovering; and, in other instances which terminated fatally . . the bile ducts have sometimes been found quite pervious and arranged in a perfectly natural manner". He goes on to describe some cases of neo-natal jaundice, and says that such cases "are apt to be met with in several successive children of the same parents".

Even more thoughtful is the comment of Goodhart (1883), who was the first, and possibly the only, person to refer to the accounts of Pearson and Cheyne. He says: "Other cases of congenital occlusion of the ducts appear to be due to malformation, and have been described by Cheyne and Underwood [i e Pearson] so long ago as 1805 and by various authors since as showing an inveterate tendency to transmission by occurring in several children of the same family It is, however, possible that such children may have all been exposed to some particular taint, such as *that of syphilis, for example, and thus the recurrence of* similar disease in succeeding infants." The nature of the "taint", however, was not recognized, though it had been understood by this date in the similar condition in foals Despite Goodhart's comments and three more descriptions being published shortly afterwards (Ashby, 1884; Hatfield, 1889, Thomas, 1891), the century ended much as it began from the point of view of neo-natal jaundice. As witness of this, Ballantyne's well-known text-book of 1902 may be quoted; on the topic of congenital obliteration of the bile-ducts he speaks of "the occurrence of family prevalence, often to a very remarkable degree; for as many as seven or even ten cases of infantile jaundice due to this lesion of the *bile-ducts have been observed in one family". Nevertheless,* he cannot have been very confident about "this lesion", for on the next page he says: "In one group of cases (usually those in which death has occurred early) the ducts may seem to the naked eye to be little if at all involved. . . ."

The Twentieth Century

Several descriptions published in the early part of this century very possibly referred to cases of icterus gravis

ception the five principal features of the condition as follows. "(1) *Its appearance in successive children of the same parents* (2) The rapid development within a few hours of birth of a jaundice which quickly became profound. (3) The occurrence of a generally drowsy condition which usually ended in convulsions, terminating fatally. (4) The severe anaemia which became more and more marked as the colour of the jaundice waned, and which persisted for a lengthy period in those affected children

Hydrops Foetalis

The earliest account of a condition that may well have been the hydropic syndrome of haemolytic disease of the newborn is that of Louyse Bourgeois published in 1609. This lady was evidently the midwife of Marie de Medici, and much in demand in Court circles. She first published her reminiscences in 1609 at the age of 46.[11] In them is an account of the delivery of a pair of twins: a stillborn girl who was hydropic, and a boy who survived for some time with marked jaundice. It is tempting to regard this account, a translation of which is given in the appendix,[12] as the earliest description both of hydrops foetalis and icterus gravis, and of the unusual combination in twins

Another account published shortly afterwards in 1614 by Felix Plater is sometimes quoted as a possible case of hydrops foetalis; a translation is offered in the appendix.[13] Other early descriptions which might include genuine cases of hydrops foetalis are those of Dorstenius (1685) and Schurig (1732), but the remaining seventeenth-century and early eighteenth-century accounts are inadmissible.[14] These, and a number of other early accounts, are referred to in Ballantyne's masterly review of the subject in 1892. Unfortunately, of the sixty-five reports in Ballantyne's list of references, rather more than twenty are in inaccessible volumes, and of the forty-two that have been examined, scarcely a dozen can be retained in the light of present knowledge as possible *cases of haemolytic disease* of the newborn[15] However, Ballantyne clearly established the [illegible]

Several reports of hydrops foetalis appeared after Ballantyne's survey, those of Seifart (1899), Andrews (1901), King (1908), Schridde (1910) and Rautmann (1912) being readily accessible. The subject was reviewed by Capon in 1922, and again by Hartmann (1928).

THE UNITY OF THE SYNDROMES

By the beginning of the twentieth century, the clinical features of both the principal syndromes of haemolytic disease of the newborn—hydrops foetalis and icterus gravis neonatorum—were clearly recognized and established. Another twenty years were to pass before any relationship between these two syndromes was suspected This delay may seem a little surprising in view of Auden's hint in 1905 (see page 19), but there is one extenuating feature

the fourteen consecutive infants in Arkwright's less con-

served to demonstrate the pathological unity of the two syndromes But the link was not at this time forged It is true that several nineteenth-century authors commented on the association of hydrops foetalis with some disorder of the haemopoietic system. Likewise, Hutchinson, in the Goulstonian lectures of 1904, drew attention to the frequent association of jaundice and congenital anaemia, and expressed the view that the two sprang from a common

ed to,

riting

com-

case

to Auden's series; when these authors returned severally to this topic they drew attention to the increased extra-

Hydrops Foetalis

The earliest account of a condition that may well have been the hydropic syndrome of haemolytic disease of the newborn is that of Louyse Bourgeois published in 1609. This lady was evidently the midwife of Marie de Medici, and much in demand in Court circles. She first published her reminiscences in 1609 at the age of 46.[11] In them is an account of the delivery of a pair of twins: a stillborn girl who was hydropic, and a boy who survived for some time *with marked jaundice. It is tempting to regard this account*, a translation of which is given in the appendix,[12] as the earliest description both of hydrops foetalis and icterus gravis, and of the unusual combination in twins.

Another account published shortly afterwards in 1614 by Felix Plater is sometimes quoted as a possible case of hydrops foetalis; a translation is offered in the appendix.[13] Other early descriptions which might include genuine cases of hydrops foetalis are those of Dorstenius (1685) and Schurig (1732), but the remaining seventeenth-century and early eighteenth-century accounts are inadmissible.[14] These, and a number of other early accounts, are referred to in Ballantyne's masterly review of the subject in 1892. Unfortunately, of the sixty-five reports in Ballantyne's list of references, rather more than twenty are in inaccessible volumes, and of the forty-two that have been examined, scarcely a dozen can be retained in the light of present knowledge as possible cases of haemolytic disease of the newborn.[15] However, Ballantyne clearly established the association of hydrops foetalis with multiparity, its familial incidence, and the properties of prematurity and bloodlessness in the infants affected by it.

Several reports of hydrops foetalis appeared after Ballantyne's survey, those of Seifart (1899), Andrews (1901), King (1908), Schridde (1910) and Rautmann (1912) being readily accessible. The subject was reviewed by Capon in 1922, and again by Hartmann (1928).

THE UNITY OF THE SYNDROMES

By the beginning of the twentieth century, the clinical features of both the principal syndromes of haemolytic disease of the newborn—hydrops foetalis and icterus gravis neonatorum—were clearly recognized and established. Another twenty years were to pass before any relationship between these two syndromes was suspected. This delay may seem a little surprising in view of Auden's hint in 1905 (see pa
about many
itself, is not
time. This fe
syndromes ir
described a family with nine consecutive jaundiced infants but with no case of hydrops; and the story is similar with the eleven icteric infants in Ritchie's family (1912-13), and the fourteen consecutive infants in Arkwright's less convincing family (1902). In the same way, some families (for example, those of Ballantyne, 1892, and of Oberndorfer,

true that several nineteenth-century authors commented on the association of hydrops foetalis with some disorder of the haemopoietic system Likewise, Hutchinson, in the Goulstonian lectures of 1904, drew attention to the frequent association of jaundice and congenital anaemia, and expressed the view that the two sprang from a common

medullary erythropoiesis in their cases (Buchan and Comrie, 1909. M'Gibbon, 1912-13). But the association between icterus gravis and hydrops foetalis still eluded the observers

In 1921, however, von Gierke, in describing the pathological features of a case of kernicterus, commented on the presence of marked erythroblastosis comparable with the descriptions by Schridde (1910), and Rautmann (1912) of that finding in hydrops foetalis; he concluded that hydrops and kernicterus might have a common origin.

Thereafter, observations on these lines began to accumulate and the association between the two syndromes was noticed and described by Capon (1922), Eichelbaum (1923), Fordyce and McAfie (1924), Plaut (1927), De Groot (1927), De Lange and Arntzenius (1929), and in 1931 the subject was fully discussed by Buhrman and Sanford, by Pasachoff and Wilson and by Salomonsen. In this year, too, there appeared a full pathological description of six cases by Ferguson, in which he says· "the cases described in this report when considered as a group, are probably representative of a definite disease entity of the newly born, and whatever the etiology may be, the underlying cause is undoubtedly the same in each instance whether or not the individual case is characterized by jaundice or edema, or whether both jaundice and edema are lacking". The unity of the syndromes was thus well established by the time of Diamond, Blackfan and Baty's full review of 1932.

Iso-Immunization

The idea that a mother might become immunized against foetal or placental tissues is nearly as old as the concept of antibodies themselves Both Landsteiner and Veit (1900) had demonstrated certain physico-chemical differences between the blood of mother and child, and Halban in 1900, the same year as the human blood groups were first defined, discussed the serological relationships of the blood of the mother and child in a paper entitled. "Agglutinationsversuche mit mütterlichem und kindlichen Blute." As a result of his experiments, he suggested not only that an exchange

of antibodies between the two was possible, but also that reciprocal active immunization might be responsible for the isoagglutinins and isolysins normally found in the serum[18] Halban, therefore, in 1900 seems to have been the first to postulate maternal iso-immunization against foetal tissues.

This idea was evidently of particular interest to those studying eclampsia It had already been observed that patients dying of this condition were found to have fragments of chorionic villi in the lungs. Weichardt (1901) suggested that the mother normally produces antibodies to neutralize toxic substances released by the foetus, and that failure to evolve such antibodies led to eclampsia in the mother Some experimental support for this view was sought by Ascoli (1902), Scholten and Veit (1902) and [illegible]

cells was clearly under consideration.

In the following year Veit summarized the problem slightly differently in saying: "when chorionic elements [illegible]

These two phenomena, the blue staining of the urine and the post-partum rise in titre of maternal agglutinins, were not invariably found, and Dienst concluded that they were pathological features of eclampsia, and so attributed this disease to direct transfusion of incompatible red cells from the foetus to the mother.

Independently, Anderson and Rosenau (1908) wrote: "It occurred to us that either the blood or protein substances in solution from the fetus or placenta may first *sensitize the* mother A subsequent introduction into the system of the mother of a similar substance may explain the convulsions and symptoms which occur in a certain class of toxaemias of pregnancy." Their anaphylactic experiments led them to incriminate the placenta rather than the foetal tissues. The subject was reviewed by Murray (1910), who was unable to support the views of Anderson and Rosenau, or of Dienst, and since the latter had by this time retracted his explanation for the cause of eclampsia, the work on iso-immunization seems to have fallen temporarily into abeyance.

It is not quite clear what awakened interest in the subject again—it may have been a light paper by Chavasse[17] in 1921—but no less than four papers on blood group iso-immunization appeared in 1923. In all four the possibility that maternal immunization could cause eclampsia was considered, and Williams specified that "the only foreign proteins present during pregnancy should be those inherited from the father through the spermatozoon and reproduced in the foetus". Neither Gruhzit nor McQuarrie made any new points, but the latter's paper stimulated Ottenberg, who had done some work on the subject several years earlier, but did not publish any results when he became aware of Dienst's work. He ends by saying: "Finally, it seems possible that several unexplained diseases, particularly jaundice of the newborn . . . may be due to accidental placental transfusion of incompatible blood." Admittedly the sentence is ambiguous in embracing also the lysis of maternal cells by the offspring's antibodies, but it is the first time that neo-natal jaundice is considered as a sequela of iso-immunization

This idea is developed further by Lenart (1928), Lenart

... though the eclampsia ... e, Allen, ... was not ... , at this time on the iso-lysins and iso-agglutinins revealed by testing foetal and maternal blood against each other. The papers of Pollitzer (1924), Hirszfeld and Zbrowski (1925), Bouchet (1926), Doghotti (1926), Mitchell (1928), ...

... therefore not unreasonable to suppose that the remains of foetal red cells may reach the mother in an antigenic state." Jonsson (1936) adopted a rather similar view after studying maternal iso-lysins in the puerperium

The next step came from reasoning about haemolytic disease, and by this time the relationship of hydrops foetalis, icterus gravis and congenital anaemia was fully appreciated. In 1938, Darrow, who appears to have been unaware of the views of Dienst or Ottenberg, extensively ...

... one may reconstruct the etiologic events as follows the mother is actively immunized against fetal red cells or some component of them The immunization may conceivably occur as a result of an accident within the placenta whereby the fetal cells or their hemoglobin gain entrance to the maternal blood sinuses The antibodies formed in ... ld through

antigenically distinct from the adult form, the story is here complete.

In the following year, however, the idea of maternal immunization against foetal tissues was again advanced seemingly *de novo*, this time to account for a transfusion reaction (Levine and Stetson, 1939). The patient, having just been delivered of a stillborn child, received blood from her husband (they were both of Group O), and suffered a severe reaction. Her serum was found to contain an un-

[illegible]

differ in any material way from those previously reported by Parr and Krischner (1932), Stetson (1933), Johnson and Conway (1933), Culbertson and Ratcliffe (1936), Zacho (1935) and Mandelbaum (1939), in which reaction to transfusion (the first transfusion in several cases) had followed recent parturition or miscarriage. Indeed, the frequent appearance of transfusion reactions in young women recently delivered of infants was, at this time, exciting comment (Wiener, 1939), and in many of these cases agglutinins could be demonstrated However, Levine and Stetson recognized that the immediate and severe response of their patient, who had not previously been transfused, suggested that she had been immunized before the transfusion against an antigen to be found in her husband. They say: "In view of the fact that the patient harboured a dead fetus for a period of several months, one may assume that the products of the disintegrating fetus were responsible for . . the isoimmunization. Presumably the immunizing property in the blood and/or tissues of the fetus must have been inherited from the father."

The importance of Levine and Setson's case was partly due to its appearance at the time when the story was about to move into its final stages In the following year Landsteiner and Wiener, preparing antisera in rabbits against

Wiener and Peters (1940) realized that some haemolytic

reaction in the case he had reported in the previous year (Levine and Katzin, 1940). Finally, the problem of the ... clearly estab- ... tigen was ... the new-

Only two further points in the development of our know-

ABO groups By far the majority of cases, however, are caused by antibodies against the Rh antigen D. The other

CHAPTER II

THE DISEASE IN MAN: HAEMOLYTIC ANAEMIA

Iso-Immunization

The cause of haemolytic disease of the newborn is iso-immunization of the mother against the red cell antigens of her foetus The origin of the iso-immunization is less clear. An obvious cause is incompatible blood transfusion, and the growth of transfusion practice in the 1940's served not only to focus attention on haemolytic disease but also to increase its incidence. However, a substantial number of cases of this disease arise apparently without the artificial stimulus of blood transfusion, and in these the mechanism of the iso-immunization remains largely undetermined.

Any explanation of the mother's immunization should take into account certain features of the clinical and family history in haemolytic disease which have been established after a decade of study These features may be considered as four in number, if consideration is confined to immunization due to the Rh antigen D, and to those cases in which it seems reasonably certain that no incompatible blood has been administered to the mother either intramuscularly or intravenously.

In the first place, it is usual for the earliest children in the family to escape the disease, sometimes the first alone is spared, in other families several children escape, but it is the first-born that may count on escape This rule is not invariable, Anderson (1953), for example, reports a recent exception and refers to others, but the finding is sufficiently constant to imply that, without the aid of blood transfusion, it is not normally possible for the process of iso-immunization, together with the resultant clinical changes in the baby, to take place during the course of a single pregnancy.

Either, therefore, the first pregnancy or some abnormality associated therewith affords an opportunity for transplacental immunization but not sufficiently early to affect the [illegible]

The second observation is a corollary of the first; just as the first-born usually escapes, so no rhesus positive infant that is born to the same parents after the first affected child [illegible]

very difficult to account for these observations.

The third feature of importance in considering iso-immunization is its low incidence. Neither the incidence of iso-immunization in rhesus negative mothers, nor that of haemolytic disease is exactly known. The difficulties are that both states can easily pass undetected, and, since they are rare, extremely large populations would have to be examined, with [illegible]
accurate result [illegible]
Jakobowicz, Gr[illegible]
illustrate this p[illegible]
the various figures that have been published that iso-immunization results in only a very small proportion of [illegible]

CHAPTER II

THE DISEASE IN MAN: HAEMOLYTIC ANAEMIA

Iso-Immunization

The cause of haemolytic disease of the newborn is iso- [illegible] ens of [illegible] clear. [illegible] n, and the growth of transfusion practice in the 1940's served not only to focus attention on haemolytic disease but also to increase its incidence. However, a substantial number of cases of this disease arise apparently without the artificial stimulus of blood transfusion, and in these the mechanism of the iso-immunization remains largely undetermined.

Any explanation of the mother's immunization should take into account certain [illegible] history in haemolytic dis[illegible] after a decade of study. [illegible] been administered to the mother either intramuscularly or intravenously.

In the first place, it is usual for the earliest children in the family to escape the disease; sometimes the first alone is spared, in other families several children escape, but it is the first-born that may count on escape. This rule is not [illegible]

anism that is suggested. Admittedly this explanation merely poses another problem: why does retroplacental haemorrhage occur? But, at least, it serves to define the nature of the enquiry.

It is reasonable to suppose that the mechanism postulated for the maternal iso-immunization against the Rh antigen D applies also to the rarer occasions of immunization against other Rh antigens and blood group antigens generally. If Mollison's (1956 *a*) calculations on the relative

random transfusion

Iso-immunization against the A and B blood groups, however, poses rather different problems from those associated with the Rh antigens. In the first place, in heterospecific pregnancy (when the mother is of Group O, and the

alteration in the quantity or qualitative character of the maternal antibody in the early post-partum period, and even then only "secretor" infants are involved. It appears that the stimulus takes place during parturition, but that the soluble blood group substance may be a more effective antigen than the intact red cell (Zuelzer and Kaplan, 1954). The incidence has been estimated as 0.8% (Vaughan and Sotos, 1955).

Clinical Aspects

Once iso-immunization is established, the maternal antibody passes freely to the foetus where it brings about some degree of destruction of the red blood cells. It may be that

mother or child in those families that are affected, but without success.

Another theory to account for the low incidence is that the only rhesus negative mothers who are liable to isoimmunization are those who are themselves offspring of rhesus negative mothers; whereas those who were conceived by rhesus positive mothers might have acquired immunological tolerance *in utero* against the rhesus positive antigen, and so would subsequently be able to tolerate their offspring's antigen without being themselves immunized (Owen, 1956) This theory, though it fits quite w... tically, has not so far been supported ... and it leaves unexplain... the foetus, a problem ne... the reverse

The fourth observatio... about Rh immunization bears on the third in being concerned with the incidence of this state. It is found that Rh immunization is significantly more common in matings that are compatible in ABO grouping than in those that are not so. This suggests that in the latter circumstances the presence of the naturally occurring anti-A and anti-B tends to eliminate either the foetuses (by abortion) or the invading foetal red cells that are incompatible in ABO grouping, before the Rh substance has had the opportunity to exert any significant antigenic effect. Whether the effect is upon the implanted foetus or upon the invading red cells is not determined; there is some statistical support for the former view, but not yet enough If the latter were shown to be the mechanism, and a little support can be found in the report of Zuelzer and Kaplan (1954), it would indicate that the antigenic stimulus was brought about by intact red cells on the maternal side of the placental barrier, and so narrow the field of enquiry to the way in which the foetal cells reach the maternal circulation

The simplest explanation of how the foetal cells immunize the mother, and one that is reasonably consistent with the four observations described, is that haemorrhage from the foetal side of the placenta may lead to the entry of foetal blood into the maternal uterine sinuses at the time of parturition That this can happen was suggested by Wiener (1948) and demonstrated by Chown (1954, *a* and *b*). This

24 hours; it is commonly very pronounced, and is responsible for the usual title of this syndrome: *icterus gravis neonatorum*. The jaundice and anaemia are independent of each other and probably spring from different origins, so that their severity and progress may be unrelated The grave neurological sequelae of the jaundice are discussed in the next chapter

more than a few days; the influence of such a phase upon prognosis has not certainly been assessed The liver is usually, and the spleen is invariably, enlarged, both are usually palpable in all stages of this syndrome Ecchymoses, and sometimes more significant evidence of haemorrhage,

during the first two or three days.

other effects are exerted by the antibody, but most of the clinical findings can be referred to anaemia and its consequences.

In severe cases the brunt of the antibodies' attack is borne *in utero*, so that the infant is either stillborn, or dies within a few hours of birth; delivery is usually premature in these cases. The principal clinical feature of this form of the disease is oedema, which is commonly diffuse and pronounced, though in rather less severe cases the oedema may show a regional distribution This oedematous form of the disease has for many years been called *hydrops foetalis*. Oedema is also pronounced in the internal organs, and may be accompanied by massive effusions, sometimes blood-stained, or general anasarca, in a very few cases the oedema may be absent, at any rate by external observation. Maceration of varying degree may be seen depending upon how long the foetus has remained *in utero* after death. Jaundice is not a feature of these cases. Extreme pallor due to marked anaemia is constant, and enlargement of the liver and spleen (which is not always easy to detect clinically, because of the softness of the oedematous tissue) is invariable Petechiae may be seen The placenta is enlarged, oedematous and friable. About 30% of infants suffering from haemolytic disease of the newborn succumb *in utero*. The differential diagnosis of hydrops foetalis must take into [illegible]

sometimes prematurely, with some degree of anaemia which is bound to worsen in the neo-natal period until the maternal antibody is eliminated from the infant's circulation, or is at any rate much reduced in quantity. In very mild cases, little more is seen than the pallor and lethargy attributable to the anaemia, which itself may worsen for two or three weeks for reasons discussed elsewhere. The chief danger in these cases is that the condition may be overlooked for about three weeks, when the infant may be found to be suffering from a severe and possibly mortal anaemia. The incidence of this form of the disease, which

bush, 1951), but neither this, nor the cord blood bilirubin level can be depended on to forecast the risk from kernicterus. The outlook is always worse in premature infants

[illegible]

is not yet confirmed

HAEMATOLOGICAL ASPECTS

The anaemia which is the principal characteristic of haemolytic disease of the newborn is superimposed on what is already a dynamic pattern of haematological readjustment found in the normal infant following birth The postnatal physiological changes have been appreciated only in recent years when some of the factors that modify the [illegible]

particularly of the lungs, and ascites. In milder cases, the child becomes increasingly lethargic, and is at all times difficult to feed; vomiting is common.

This syndrome of icterus gravis is subject to much varia-

emphasized that a noticeable and sometimes pronounced

weak or negative. There are other features found when the condition is due to anti-A or anti-B but not in the Rh-induced disease, namely spherocytosis and an increased

the degree of anaemia that may develop (Mollison and Cut-

to anaemia is not confined to the premature infant but is a

row erythropoiesis through anoxia, works well enough in the normal infant, but that in infants who are premature or anaemic from other causes, recovery is delayed partly because of the sluggish marrow response, and partly because their rapid growth calls for an even greater rate of erythropoiesis than normal.

The anaemia of haemolytic disease of the newborn

5 millions per cu mm. (Marks, Gairdner and Roscoe, 1955). After an initial rise on the first day, these values steadily fall, until about the sixtieth day. This decline in haemoglobin concentration and red count has been clearly divided into two components by Gairdner, Marks and Roscoe (1952), who included a study of the bone marrow in their investigations. The initial component of the decline [illegible] dis-

[illegible] bin after [illegible] oft, 1946) Since the oxygen carrying power of each red cell is suddenly improved at birth, a number of red cells are redundant, and there is no call on the marrow to replace them

The second component occupies the period from the [illegible] during this time the erythro- [illegible] eadily increases, the eryth- [illegible] r cu. mm. to 25,000. The [illegible] inues to fall, however, because [illegible] n weight and circulatory volum [illegible] when [illegible] ner *et* [illegible] portin [illegible]

sponse to anoxia appears to be rather sluggish, so that the

coe (1955) show that the relative insensitivity of the marrow to anaemia is not confined to the premature infant but is a general characteristic of early infancy and may be mitigated by the exhibition of cobalt. It seems, therefore, that the delicately adjusted mechanism which maintains the haemoglobin concentration at about 11G, by the control of marrow erythropoiesis through anoxia, works well enough in the normal infant; but that in infants who are premature or anaemic from other causes, recovery is delayed partly because of the sluggish marrow response, and partly because their rapid growth calls for an even greater rate of erythropoiesis than normal

The anaemia of haemolytic disease of the newborn

show that the Rh antibody, or perhaps some forms of it, may attack immature as well as formed

immature cells are also attacked, and hence the marrow response appears suppressed. This might explain why it has never been easy to relate the degree of erythroblastaemia to the clinical severity of the condition.

The onset of the anaemia *in utero* is no doubt also responsible for the macrocytosis that is characteristic of haemolytic disease due to Rh incompatibility. The anoxic stimulus *in utero* calls forth red cells containing foetal-type [illegible] corded in this condition (Reisner, 1943) may also be a reflection of the same circumstances.

In haemolytic disease due to anti-A or anti-B the red cells apparently show rather different properties (Levine *et al.*, 1953, Crawford *et al.*, 1953; Shumway *et al.*, 1955) The cells are rather smaller than normal, and spherocytosis may be pronounced. Their osmotic and mechanical fragility, which is usually normal in the Rh-induced disease, is not only increased in that due to anti-A, but also shows a more abrupt change over the range of saline dilutions. Reticulocytosis and erythroblastaemia are marked.

Histological Aspects

The dominant feature in the microscopical examination of the infants' tissues, and one which springs from the anaemia, is marked extramedullary erythropoiesis. It is seen principally in the liver and spleen, and also in lymphoid tissue elsewhere in the body, and in the kidney, suprarenals and placenta Large numbers of erythroblasts and normoblasts are seen both in these sites and in the [illegible]

though variable in degree. The parenchymal cells may be shrunken, and the sinusoids dilated (Craig, 1950), and there may be disorganization of the hepatic columns, fatty change in the cells and granulation of the cytoplasm (Potter, 1947) Pigment and iron, generally having a similar distribution, are seen in the parenchymal cells, chiefly in the periphery of the liver lobule; the Kupffer cells may not contain iron even when the parenchyma is impregnated (Craig, 1950).

Together with, and possibly associated with, the damage to the liver parenchyma, there is in icterus gravis, evidence of biliary retention The bile canaliculi are distended and bile-thrombi may be seen both in these and in the larger vessels

Other changes, probably less specific, have been described, though not yet confirmed, in large series of cases The increase in the pancreatic islet tissue is also seen in children of diabetic mothers (Miller, Johnson and Durlacher, 1944) A more rapid differentiation of the chromophobe cells of the pituitary has been reported (Ranström, 1951), but this change also is seen in the offspring of diabetic mothers Changes in the suprarenals have been described as specific to the hydropic syndrome since the paper of King (1908) Gilmour (1944) believes that lipoid infiltration of the adrenal cortex may be found only in hydrops foetalis; whether this difference from icterus gravis is one of degree rather than kind, and whether this may be a non-specific response at this stage of foetal development, are problems only to be resolved by the study of a larger series of cases Gilmour, among others, also draws attention to an abnormality of the line of calcification at the osteochondral junction of long bones, which is sometimes observed in hydrops foetalis.

The pathological changes in haemolytic disease of the newborn are not specific to the condition An important point made by Morison (1952) is that the tissues in this condition all appear less mature than would be expected in a normal infant of corresponding age.

CHAPTER III

THE DISEASE IN MAN: KERNICTERUS

Historical Aspects

In haemolytic disease of the newborn, jaundice is a [illegible]

infants during the first week, and also to the patchy distribution in the brain of yellow-stained areas which are found in infants that die of the condition. The latter, the brain lesions, were described before the syndrome had been differentiated specifically from the mass of writings about convulsions and other nervous disorders in childhood.

Kerckring's (1670) autopsy account of a severely jaundiced eight-month foetus does not include a description of the brain, but that of Wrisberg (1764) contains the sentence · "In basi cranii varia loca, ut lateralium sinuum tractus, pars maxima cavitatis frontalis, ad ductum ossis petrosi alibique pigmento illo tingebantur croceo." Though open to several interpretations, the description is surely consistent with a patchy yellow pigmentation of the brain. It is necessary to pass over the description of Kirronosis by Lobstein[18] (1826), which is a different condition, and come to what is possibly one of the earliest accounts of kernicterus, that of Billard in a text-book of children's diseases, published in 1828. The account, in Stewart's translation,[19] runs as follows:

"I have remarked the yellow colouring which constitutes jaundice, in four instances, in the brain and spinal marrow;

the brain, which was of moderate firmness, presented a uniform and bright yellow appearance in two of these subjects, while the colour was in isolated patches in the other two. In three of these cases the substance of the medulla [illegible]

It seems likely that this appearance was reasonably familiar to observers in the early nineteenth century. Both Otto (1830) and Dugès (1832) mention it briefly, and Hervieux (1847) most specifically describes the pigmentation as being "tantôt restreinte à certaines parties comme la [illegible]

It was not until 1903, however, that a full clinical and pathological description was given, and it was in this account that Schmorl coined the word kernicterus. He carefully distinguished between a uniform surface impregnation of the brain by bile pigment, a condition generally considered nowadays to result most commonly from a post-mortem change, and the staining of the basal ganglia alone, [illegible]

foetalis he proposed a [illegible]

hence of maternal blood-group iso-immunization

Almost immediately, however, doubts were raised by the paper of Forster and McCormack (1944), which reported a case of kernicterus that did not appear to be associated

with haemolytic disease of the newborn. From several other

cations of Dublin (1949, 1951), Aidin, Corner and Tovey (1950), Zuelzer and Mudgett (1950), Butler and Spector

The Origin of the Condition

Icterus Neonatorum

Jaundice in the newborn is central to the problem of

fied and how it is excreted is not clear. Czerna and Liebmann (1923) held that the bilirubin level in the umbilical artery was higher than that in the veins and hence that this

If the bilirubin is not excreted it may be converted to urobilinogen which may either be excreted transplacentally (Winternitz, 1926) or some of it, at any rate, may find its way into the amniotic fluid Bevis (1952*a*), using a paracentesis technique in the ante-natal period, detected urobilinogen in the amniotic fluid He agreed that the urobilinogen was of foetal origin, but that intestinal bacteria could not have played any part in its conversion in the circumstances

Coquoin-Carnot and Pignard, 1955), and it is known to have a rapid turn-over; it is against such a background that

estimations of foetal products in this liquid must be viewed. However it may be arranged, the disposal of bile pigments *in utero* seems to be satisfactorily accomplished by the foetus.

[illegible]

blood of the normal infant so that the concentration rises from about 1·5 mgm. per 100 ml to between 5 mgm. and 10 mgm in the majority of cases, on the second or third day.

[illegible]

impaired, and the accumulation is also favoured by the fact that the renal threshold for bilirubin is generally high in

[illegible]

man is not clear, though in rats there is some evidence of haemolysis (Allison, Moore and Sharman, 1956).

In newborn infants the serum bilirubin level needs to be very much higher for clinical jaundice to result than is usual in adults (Larsen and With, 1943; Biering-Sørensen and With, 1946). Hence, even mild jaundice reflects a markedly raised serum bilirubin. There is, of course, no absolute level *of bilirubinaemia that determines the clinical appearance* of jaundice (Davidson, Merritt and Weech, 1941), and indi- [illegible]

[illegible]

function is the principal factor, the circumstances are sometimes pathological, but are in many cases thought to be due to a physiological immaturity of the liver; hence, these cases, too, can be classed as "physiological jaundice". And no one can say in an individual case of physiological jaundice which of the two factors predominates, unless extensive technical investigation is undertaken, and not always then.

Thus a substantial proportion of newborn infants suffer icterus *neonatorum, often without handicap, either through* blood destruction or through hepatic insufficiency, or a combination *of both these factors There is therefore* a difference in degree only between the fleeting jaundice of icterus *neonatorum and severe haemolytic disease of the* newborn with kernicterus, the two aforementioned factors playing *their respective and related parts.*[20]

Increased Blood Destruction

Of the two factors that contribute to icterus neonatorum the rate of blood destruction is the more easily *measured.*

It is now clear from the work of Findlay (1946), Mollison (1948), Hedenstedt and Vahlquist (1948) and Gairdner

nancies. There cannot, of course, be any close correlation in these matters because of the other variable, hepatic efficiency. There seems little doubt that when there is immune destruction of red cells, the process is initiated before birth, so that there may be some degree of hyperbilirubinaemia at birth, which becomes rather more pronounced and reaches its peak earlier than in the normal infant

The breakdown of foetal red cells leads, in Bevis's (1952 *b*) view, to a rise in non-haematin iron in the amniotic fluid The excess of iron, so derived, might act as a tissue poison and inhibit the placental catalase, which may be responsible for haemoglobin catabolism and so divert haemoglobin breakdown into abnormal channels.

Once the infant is born, the rate of red cell destruction

1

is normal.

Liver Function

[illegible]

means, but these have proved of limited value, both because [illegible]

(e.g. laevulose tolerance, Thompson and Wilkinson, 1940, and bromsulphthalein excretion, Obrinsky, Denley and Brauer, 1952) are very indirect means of studying the specific action of hepatic function that is concerned, namely bile pigment metabolism However, the last authors did find a reduced bromsulphthalein excretion in premature infants correlating rather with age than with weight. Attempts to assess liver efficiency in disposing of bile pigments after intravenous injection of bilirubin into newborn infants have given rather equivocal results (Lin and Eastman, 1937)

The difficulties in assessing liver activities have led to the argument that the hepatic dysfunction is simply a physiological immaturity, and that it could be examined by comparing the bilirubin disposal between premature and full-term infants during the first week of life. Such studies have, in general, shown that in premature infants hyperbilirubinaemia is more common, more pronounced and more prolonged than in full-term ones, though the influence of heterospecific pregnancy has not always been excluded. Billing, Cole and Lathe (1954) express the view that the hyperbilirubinaemia is related more to immaturity measured by weight than that assessed by age. They suppose that the hepatic dysfunction is quite specific, namely a failure to convert the indirect-reacting van den Bergh pigment, which they think may be toxic, to the less toxic direct-reacting pigment This lays all the blame on liver insufficiency, and the strain would be greater were there any increase in blood destruction. This concept also assumes that the bile pigment conversion to which they refer is normally carried out by the foetal liver during the last part

of pregnancy. The suggestion that simple immaturity is the principal cause of hyperbilirubinaemia (though not unaided, according to Obrinsky *et al.*, 1954) fits well with the observations that kernicterus is notably associated with prematurity, and that haemolytic disease of the newborn, even in full-term infants, is characterized by an appearance of immaturity of the tissues.

The supposition that immaturity of the liver is the hepatic factor leading to hyperbilirubinaemia is not universally accepted Emery (1952, 1953), for example, suggests that a rapid involution of the left lobe of the liver during the first five days, perhaps due to circulatory readjustment after birth, may be the cause of a decline in liver activity. Another view, fostered by Baar (1945, 1946) and Parsons (1947), is that kernicterus differs not in degree but in kind from simple icterus neonatorum, and that in the former condition the liver is certainly damaged so that the brain lesions are associated with positive liver damage in a manner comparable with Wilson's hepato-lenticular degeneration Wilson (1912) himself commented on the similarity of this condition with kernicterus, though he also discussed their differences Moreover, there is a certain amount of evidence in man and experimentally in several animal species that damage to the liver may be associated with damage to the brain.[21]

This point has been taken up recently by Polani (1954 *a* and *b*) in experiments of hetero-immunization on young rats He tried to produce an equivalent degree of anoxia by three means oxygen privation, the administration of a rabbit anti-rat cell haemolytic serum, and the administration of phenylhydrazine which would be haemolytic through its physical properties The haemolytic serum produced hepatic lesions consistent with those seen in haemolytic disease of the newborn, and Polani tentatively concluded that since neither pure anoxia nor the products of phenylhydrazine haemolysis provoked such typical lesions, the damage might have resulted from the direct action of the antibody Furthermore, the brain lesions were characteristic of kernicterus only in those animals receiving haemolytic serum, and though some animals suffered

serum. These experiments being conducted on the rat and being concerned solely with post-natal damage may not reflect the situation in man, but they offer some support to the association of liver damage with cerebral lesions. The effect of the antiserum, being derived from another species, may easily be more toxic to the liver cells than an iso-

[illegible] obscure.
[illegible] gold and
Wood (1954), in which liver damage, indistinguishable from that associated with haemolytic disease of the newborn, resulted from hepatitis probably of viral origin, serve as a reminder that the hepatic changes in haemolytic disease are not specific to this condition. The liver damage in haemolytic disease of the newborn in man and in other animals could be ascribed to the iso-antibody. One objection, that the Rh antigen might not be present in liver cells, may

[illegible]

objection, that there is no antibody present in kernicterus due to prematurity, could be evaded by falling back on the view that in these cases the liver is not damaged but merely physiologically immature But without additional support it is not easy to attribute the liver damage to antibody

[illegible]

simple anoxia as the cause, and there remains a possibility that some toxic agent other than antibody may be concerned; in view of the clinical evidence of toxicity and the brain damage found in kernicterus, this suggestion warrants

further examination. The agent that leaps to mind is the pigment that stains the brain in kernicterus.

Rimington [illegible]
urine of ce [illegible]
pears that t [illegible]
was very p [illegible]

[illegible]

suggested that mesobilifuscin may form from the breakdown of haemoglobin (Engel, 1940) and of myoglobin (Meldolesi, Siedel and Moller, 1939). The association of mesobilifuscin with muscle breakdown is interesting in relation to the observations of Beneke (1913) and Dollet (1939), who described an interstitial myositis, with deposition of pigment crystals, in cases of kernicterus There is, however, a little more recent evident of the association of mesobilifuscin with kernicterus Pickles (1949) detected an abnormal bile pigment in the blood of two of her cases [illegible]

[illegible]

rubin from the brains of two cases of kernicterus. In these cases evolution of mesobilifuscin would be attributed to catalase poisoning, due to the excess iron from the breakdown of red cells, which might divert haemoglobin cata- [illegible]

[illegible]

toxic substance.

Several other pigments have been suggested as the kernicteric pigment or as being very closely related. Vogel (1953) favours mesobilirubin even though it is non-toxic. The pigment he extracted from the brains of three cases of

mesobilirubin was retained for any time in the tissues, which were themselves undamaged. He suggested that his results were compatible with the theory that bilirubin on reaching the brain might, given time, be reduced to mesobilirubin and retained in the cells, but that the neuronal damage found in kernicterus was not the consequence of pigment deposition. However, the application of this evidence to man cannot be assessed.

Haematin, found in the form of methaemalbumin in the serum of cases of haemolytic disease has also been con-

symptoms of cerebral malaria. If disordered haemoglobin metabolism is a feature of haemolytic disease, it may be that haematin-like compounds are concerned. An unidentified haem pigment of this kind has been implicated by

sig-

was

This

haem pigment was not found by Abelson and Boggs in the blood of normal newborn infants; its properties are similar to those of Tuttle's methaemalbumin, though the authors

the indirect-reacting form of bilirubin by experiments on rats and rabbits; he also summarizes the difference between the several forms of bilirubin and their relationships with the various types of jaundice (Lathe, 1954 *a*).

It is clear from all this evidence that the identity of kernicteric pigment has not yet been established beyond all doubt, nor have the relationships and activities of the several suspect pigments been determined.[22] Nevertheless, bilirubin has been used from time to time to investigate the properties of the blood-brain barrier.

The Blood-brain Barrier

This term refers to the physiological properties of the endothelial cells of the capillaries supplying the brain tissue. Substances may, of course, pass from the blood into the cerebro-spinal fluid, and thence into the brain, but neither of the barriers on this route is the blood-brain barrier Besides, in jaundice only very little bile pigment enters the cerebro-spinal fluid in the adult (Amatuzio, Weber and Nesbitt, 1953) or in infants with haemolytic disease (Stempfel and Zetterstrom, 1955).

The blood-brain barrier exists in all vertebrates that have been examined. It markedly reduces the accessibility to the brain of all but a few substances; and when permeability is increased after physical or chemical trauma for example, the penetration is at first localized to certain areas, which do not, however, correspond well with the areas most frequently stained in kernicterus. The barrier retains its efficiency for about an hour after death The site of the barrier has been the subject of much speculation, but it is generally held to be very close to the capillary endothelium, perhaps the intercellular cement or possibly the ground substance of the C.N.S (Hess, 1953).

There are two important variables in the study of the blood-brain barrier, the species concerned and the substance that is being examined; the effect of bile pigments on the barrier in young human subjects can be inferred only with the greatest caution. There is some evidence, however, that in some species and using some substances the barrier is more permeable in the newborn or immature animal.

Behnsen (1927) found this to be true of mice with respect to trypan blue. Stern and Peyrot, in the same year, used sodium ferrocyanide as a dye and found this to be true, not of guinea-pigs which they judged to be born in a relatively mature state, but of rabbits, rats, mice, dogs and cats for varying periods after birth. Another index of maturity of the young animal which they found closely correlated with permeability of the barrier was the time at which the eyes became opened. Bakay (1956) found the barrier much more permeable to P^{32} in foetal and young rabbits than in adults *An entirely contrary view has been expressed* by Grontoft (1954) using trypan blue on the human foetus He believed the barrier to be entirely impermeable in human foetuses of between 5 and 30 cm. length unless it has been damaged, usually by anoxia.

Bilirubin itself has been the subject of some experimental study of the barrier in certain species Massive doses of bilirubin in animals not normally having that substance in their serum (e.g rats and rabbits) may, however, prove toxic to the barrier The same is true of the administration of heterophile antibody with bilirubin in an attempt to reproduce the environment of haemolytic disease (Dereymaeker, 1949) In man the adult cases in which bile pigment has penetrated the barrier (Rutledge and Neuberger, 1942; Sherlock, 1955) could be explained if the adenocarcinomata from which they suffered had metastasized to the brain

The relationship between immaturity of the young animal and the permeability of the blood-brain barrier fits very *well with the observation that kernicterus occurs in premature* infants and in haemolytic disease in which, as Morison has shown, the tissues are generally underdeveloped in relation to age

The Origin of Kernicterus

The evidence available at the present time permits a very tentative reconstruction of the course of events leading to kernicterus Clearly the process may start *in utero* In cases where there is increased blood destruction due to iso-immunization against any of the blood-group antigens, *there is excessive haemoglobin catabolism flooding the*

the indirect-reacting form of bilirubin by experiments on rats and rabbits; he also summarizes the difference between the several forms of bilirubin and their relationships with the various types of jaundice (Lathe, 1954 *a*).

It is clear from all this evidence that the identity of kernicteric pigment has not yet been established beyond all doubt, nor have the relationships and activities of the several suspect pigments been determined.[22] Nevertheless, bilirubin has been used from time to time to investigate the properties of the blood-brain barrier.

The Blood-brain Barrier

This term refers to the physiological properties of the endothelial cells of the capillaries supplying the brain tissue. Substances may, of course, pass from the blood into the cerebro-spinal fluid, and thence into the brain, but neither of the barriers on this route is the blood-brain barrier Besides, in jaundice only very little bile pigment enters the cerebro-spinal fluid in the adult (Amatuzio, Weber and Nesbitt, 1953) or in infants with haemolytic disease (Stempfel and Zetterstrom, 1955)

The blood-brain barrier exists in all vertebrates that have been examined. It markedly reduces the accessibility to the brain of all but a few substances, and when permeability is increased after physical or chemical trauma for example, the penetration is at first localized to certain areas, which do not, however, correspond well with the areas most frequently stained in kernicterus The barrier retains its efficiency for about an hour after death. The site of the barrier has been the subject of much speculation, but it is generally held to be very close to the capillary endothelium, perhaps the intercellular cement or possibly the ground substance of the C.N S. (Hess, 1953).

There are two important variables in the study of the blood-brain barrier, the species concerned and the substance that is being examined: the effect of bile pigments on the barrier in young human subjects can be inferred only with the greatest caution There is some evidence, however, that in some species and using some substances the barrier is more permeable in the newborn or immature animal

Prematurity is thus the *point d'appui* of kernicterus, for only in this state can be found the essential combination of liver insufficiency and permeability of the blood-brain barrier.

Clinical and Pathological Features

In acute cases the main burden of kernicterus is carried in the first week of life The onset may be quite sudden (Gerrard, 1952) on about the second day, and death commonly takes place between the third and sixth days The main features are marked jaundice and evidence of neurological disturbance A remarkable lethargy is frequent, and failure to feed is a characteristic manifestation Evidence of hypertonia is a constant finding, usually generalized rigidity, but sometimes the increased tone may be confined to a part of one extremity, opisthotonos and convulsions are common Occasionally periods of pronounced hypotonia are interspersed between spasms of rigidity. The peculiar nature of the "cerebral cry" which accompanies this condition is striking, and in the terminal stages pulmonary disorders, oedema and haemorrhage, are almost invariable (Vaughan, Allen and Diamond, 1950, Govan and Scott, 1953) Cyanosis and disorders of temperature regulation may be found It is not yet quite clear whether the symptoms of kernicterus associated with haemolytic disease differ from those in cases of simple prematurity. In both forms of the disorder the infants can be regarded as immature, and in both of them the risk of kernicterus is considerable once the serum bilirubin concentration reaches the critical level of about 18 mgm per 100 ml The rate of onset may seem to differ, being swift in haemolytic disease, usually terminating in death on or before the fourth day, whereas in prematurity nearly half the cases develop on about the sixth day, and some later (Claireaux *et al*, 1953; Crosse *et al*, 1955)

Those infants that survive the first week usually suffer permanent residual signs of neurological disorder General or local spastic rigidity, choreiform movements, deafness, mental retardation and epilepsy have frequently been de-

body with bile pigments. How far there is disordered haemoglobin catabolism with the production of abnormal and possibly toxic pigments cannot yet be established. The increase of blood destruction, however, may not be indispensable to the production of kernicterus, for some premature infants may become jaundiced and suffer kernicterus without unequivocal evidence of excessive haemoglobin breakdown. In these cases a functional immaturity of the liver is blamed for the accumulation of bile pigments, and this organ would be even more embarrassed if excessive haemolysis were superimposed. The liver insufficiency may be a simple immaturity, perhaps a specific failure to convert indirect-reacting pigment to direct-reacting pigment, and would be fostered by general prematurity, and, in the case of haemolytic disease, by the general immaturity which seems to follow the foetus' attempt to develop under the intra-uterine barrage of antibody. The liver dysfunction may be further accentuated by the liver damage invariably associated with haemolytic disease, possibly a consequence of abnormal haemoglobin catabolism providing toxic end-products. The liver failure, abetted by the other factors mentioned, causes an accumulation of normal bile pigments (perhaps bilirubin) which in excess may be toxic; in addition there may be other, probably abnormal, bile pigments or similar compounds which also may be toxic. One or more of such compounds passes the blood-brain barrier, which in the premature infant may be still ineffective, and, exhibiting an affinity for tissue cells, depresses cellular oxidation by inactivation of the cytochrome system. This effect, which may well be widespread throughout the body, finds the brain cells the most susceptible, so that these suffer first. The susceptibility of brain tissue may be due to its affinity for lipids, in which indirect-reacting bilirubin is readily soluble (Lathe, 1956). One must suppose either that damaged cells display particular affinity for the fat-soluble pigment, or that the pigment is more particularly soluble in the lipids of grey matter. The absorption of pigment by the brain cells and elsewhere may be reflected in the fall in serum bilirubin which is reported to occur in these cases shortly before death (McLean, Lacy and Harris, 1955).

ment can also been seen in the ground substance of the

seem to have lost their nuclei, but an appearance more characteristic of kernicterus is an eosinophilic change in the cytoplasm. This may occur in cells which contain no pigment, just as pigmented cells may show no morphological change (Corner, 1955). Corner also observed that

scribed. The deafness, which may itself be the cause of apparent mental backwardness in the children, may be either cochlear or nuclear, and certainly appears to be bilateral with the greatest loss among the higher frequencies (Fisch and Osborn, 1954). There is some evidence that the appearance of kernicterus as a complication of haemolytic disease exhibits a familial tendency and occurs more commonly in boys than in girls (Litchfield, 1945; Claireaux, 1950; Vaughan *et al*, 1950) A similar sex preponderance was noticed by Crosse *et al* (1955) in cases associated with prematurity, and a slight excess of second-born twins was also seen These authors observed no relationship of incidence with ABO incompatability in a series of premature infants showing no Rh immunization, but they were able to lend some support to the view that anoxia might play a contributory part in initiating the disease, a theory considered first by Beneke (1907) and returned to frequently on later occasions, particularly by Govan and Scott (1953) The incidence of kernicterus is not certainly determined[23]

The pathological findings in this disease are not specific. The patchy pigmentation of the brain is the most characteristic feature and there are at the present time few convincing accounts of kernicteric lesions in the absence of pigment However, the pigment fades after exposure to light, and so it is desirable to study the brain freshly, or to preserve it uncut until examination is convenient. There is no constancy in the distribution of the pigment in the brain except that the staining is symmetrical Certain sites are more commonly affected than others, for example, the pes hippocampi, the corpus striatum, thalamus, inferior olive and dentate nucleus It seems, however, that any part of the grey matter of the brain and the upper parts of the spinal cord may be pigmented and it is not uncommon to find zones of white matter similarly affected

The histological appearance of the brain in kernicterus is not impressive. The most characteristic feature is the yellow pigment, and this is not easily demonstrated in paraffin-embedded sections because of its solubility in the alcohol, chloroform and xylol normally used in making the preparations In frozen sections, however, the numerous

all rhesus positive people are equally rhesus positive This, however, is probably not true. A simple example is the difference between a sample of homozygous cells (XX) and a heterozygous sample (XY), the former giving the higher titre against the specific antibody (anti-X) Not all blood group systems exhibit this difference, and when it exists it can usually be allowed for. A more subtle form of variation is found with the Rh antigen D Most Rh positive persons possess the D antigen in what might be described as full measure Some, however, possess a form of antigen that reacts less well with anti-D by most serological techniques These supposedly weaker forms of D are called D^u, and this term refers to a wide variation in "D weakness" among cell samples Other blood group antigens exhibit similar variation, such as the Duffy group in man (Race, Sanger and Lehane, 1953), the A′ group in dogs (Christian, Ervin and Young, 1951), and probably the G′ group in rabbits (Kellner and Hedal, 1953) It is not certain that these variations are purely quantitative, but the explanation seems to be a reasonable one, and gains some support, for rabbit cells at any rate, from certain absorption experiments undertaken by Heard (1955 *b*), and the isotope studies of Boursnell, Heard and Rizk (1955) There seems no reason why it should not be possible to estimate the number of antigen sites per cell by isotope-marked antisera in the manner of Boursnell, Coombs and Rizk (1953), and so to indicate whether D^u cells have fewer antigen sites than D cells The degree of reactivity of D^u cells seems to be accurately heritable

The third assumption is that blood group antigens on the cell are independent of each other and all equally accessible to the specific antibodies The matter of accessibility may be illustrated by some properties of ox cells exposed to rabbit anti-ox cell sera, an immune system sufficiently similar to blood group iso-antibody action as to raise doubts whether the same characteristics may not be discovered among some blood groups Gleeson-White, Heard, Mynors and Coombs (1950) showed that such antisera agglutinated the cells from certain oxen to a titre not far short of that obtained by haemolysis, using the same antisera in the pres-

CHAPTER IV

SEROLOGICAL CONSIDERATIONS

THE ANTIGEN

The study of blood groups is the keystone to the investigation of haemolytic disease of the newborn. It is the blood group incompatibility between mother and child that is the essential feature from which this condition develops. One looks to blood group studies, therefore, to provide information about this incompatibility and, if possible, some measurement of it Unfortunately serological measurements are seldom precise and the information afforded by serological techniques is increasingly difficult to assess

Blood group studies during the first forty years of this century were almost entirely confined to the exposure of red cells, usually suspended in a saline menstruum; to the action of anti-sera, natural or induced, and the observation of any agglutination that resulted During the last decade, however, it has become increasingly apparent how limited is the information to be derived from this procedure alone, or with the assistance of differential absorptions, in the study of human and animal blood groups One might first consider, as examples, three assumptions relating to the antigen which would probably have been regarded as justifiable in the earlier part of this century. First, it has been

one excepts the circumstances, rare in man but not unusual in cattle, of an exchange of haemopoietic tissues between binovular twins *in utero*.

Secondly, it has been assumed that blood group antigens are quantitatively similar in all who possess them, e g. that

sample, whose genotype they called —D—/—D—, was in fact more reactive with anti-D sera than the usual run of D-positive cells There are other examples of one blood group antigen interfering with the expression of others, one of particular interest being the influence of the possession of A antigen in the expression of the Lewis groups (Race and Sanger, 1954; Cutbush, Giblett and Mollison, 1956).

These observations seriously diminish confidence in the usefulness of the simple agglutination reaction and have led to studies using cells that are modified either pathologically, as in cases of paroxysmal nocturnal haemoglobinuria, or artificially by treatment with proteolytic enzymes By these and other means an attempt has been made to study the action of red cell iso-antibodies

The Antibody

The classical view of agglutination supposes that, in the first stage of the reaction, antibody globulin is exclusively adsorbed on to the cell surface unaided to form a firm but reversible combination. The second stage of the reaction is said to follow this state of sensitization of the cells and to be manifest by agglutination of the cells or, if complement is present, by lysis Few of these assumptions are without exception In the first place, there is evidence that antibody globulin is not exclusively adsorbed, but that normal globulin is non-specifically adsorbed whenever cells are exposed to serum (Boursnell, Coombs and Rizk, 1953). However, it is apparently the adsorption of the antibody globulin that confers on the cells the peculiar properties associated with sensitization Secondy, it may be that not all antibodies can sensitize the specific cells unaided, for some forms of human anti-P and anti-Le[a] appear to require complement or some component of fresh serum to effect combination with the cell (Mollison and Cutbush, 1955) Finally, the second stage of the reaction may not follow sensitization. The failure of sensitized cells to lyse in the presence of complement will be considered later, but the failure of such cells to agglutinate is of more immediate interest

Those antibodies that sensitize without agglutinating,

ence of complement; but the cells of other oxen were agglutinated little or not at all by these antisera, though they

showed this property as "inagglutinable" cells, and showed

may account for the observations that in young animals the antigen may not be detectable until some time after birth (in human infants, Witebsky and Engasser, 1949; in piglets, Goodwin and Coombs, 1956), and that some pig blood groups cannot be detected by simple agglutination (Saison *et al*, 1955), but there are other explanations that could also account for these facts.

There is also some evidence that the reactivity of some

inherited as a complex of three closely associated but normally independent antigens called C, D and E (for F can be ignored for the present argument), each of which may be found in various forms, e g D, d, D^u^. Race, Sanger and Selwyn (1951) described a sample of blood in which no evidence could be found of any form of the C or E anti-

might not have become incorporated in the D antigen so that more D substance than usual might be present on the red cells. However that may be, they showed that this cell

that reported in certain other serological fields (e g. the properties of rheumatoid arthritis sera) and appeared to be an *in vitro* peculiarity, for spontaneous clumping of cells *from human infants* with haemolytic disease *is not* usually seen (though the phenomenon is common in foals and piglets with the same disease) However, it was shown that the plasma of infants less than six months of age was not effective *in vitro* in causing incomplete antibodies to agglutinate the cells (Witebsky, Rubin and Blum, 1947; Gurevitch, Polishuk and Hermoni, 1947) Another curious property is that of serum in bringing about agglutination by antisera that at first sight appear to contain incomplete antibody, described by Lewis and Chown (1954) They *found that some samples of incomplete anti-D could affect* agglutination only if normal serum was used as a testing medium, but only about 10% of randomly selected sera displayed this power, nor would these operate if applied to the cells after sensitization had taken place. This serum *effect evidently differs from the property of plasma* described earlier As far as bovine albumin is concerned, it seemed likely that the effect was due less to the albumin than to the alpha-globulin inseparably mixed with it (McCulloch, 1950). The albumin test has been used with fair success in horses, but has not been found very effective in dogs, it has had no substantial trial in pigs or rabbits

The same effect can be produced not only by plasma and albumin but also by several non-protein colloids such as 10% gelatin (Fisk and McGee, 1947), gum acacia, polyvinyl alcohol (Levine, 1946) *and* dextran (Grubb, 1949; Jones, 1950) To detect sensitization of cells by human anti-A or anti-B, Munk-Anderson (1956) uses a medium compounded of 5% dextran, 1·3% saline and 25 to 50% of serum Jandl and Castle (1956) have shown that the effect, *which is not on the incomplete antibody as such, but on the* sensitized cells, is brought about by large anisometric molecules in sufficient concentration Using cells sensitized with anti-D, or with metallo-protein complexes which evidently modify the physical properties of the cells in the same way that *immune sensitization does, they found that the de-*...g order of efficiency was whole plasma proteins,

called *incomplete antibodies*, do not differ in specificity from those that effect agglutination. Thus anti-D may be found in an agglutinating form and an incomplete form. It is true that some antibodies appear rather more commonly in the incomplete form (e g anti-D and anti-Fy[a]), whereas others are more usually agglutinating (e g. anti-A and anti-P) Moreover, antisera may contain varying quantities of the agglutinating and incomplete forms of antibody active against the same antigen And not only might the relative quantities in the mixture be unknown, but also the relative affinities of the forms of antibody for the antigen be difficult to determine It is usual for the incomplete antibody to have the greater affinity for the cell so that if it is present in the greater quantity, the action of the agglutinating antibody would be masked and it would go undetected, if the agglutinating antibody predominated in higher titre in the mixture then a prozone effect would indicate the greater affinity of the incomplete antibody for the antigen The greater affinity shown by the incomplete antibody sometimes leads to the antigen sites in the cells being so saturated with antibody that none is available for the action of agglutinating antibody so that the cells will not agglutinate even under the influence of this antibody Not all samples of incomplete antibody show this blocking effect which has little practical importance

Two significant developments in the study of incomplete antibodies have taken place in the last decade The first of these was the discovery that modifications of the menstruum in which the test was conducted allowed some incomplete antibodies to effect agglutination; and the other was the observation that a similar effect could be achieved by modifying the surface of the red cell by enzyme action. The former method, that of replacing saline as a diluent by human plasma of appropriate group, or by 20% bovine albumin, or better still, by a suitable mixture of the two, was of great importance in the detection of incomplete antibodies, though not all of them were revealed by this means. The nature of this reaction was not at first at all clear. As far as the influence of plasma was concerned, it appeared that the agglutination-enhancing effect was different from

nomenon due to bacterial infection of the blood sample. From these observations, it may appear that trypsin serves merely to render the cell non-specifically prone to agglu-

present. Moreover, some antigens that are destroyed by

normal serum components; or it may be merely that the

the result was merely a rather tenacious form of rouleaux formation. It may be, however, that in seeking to explain

tion as a not too common and not very important consequence.

With the consideration of the other method of detecting incomplete antibodies, the modification of red cells by enzyme action, one enters uncharted territory. The story originated in the observation of Pickles (1946) that red cells sensitized with incomplete anti-D were agglutinated by filtrates from *Vibro cholerae*, and in 1947 Morton and

The enzyme action is effective whether the cells are sensitized or unsensitized at the time of exposure, but whether the cells receive trypsin or antibody first, they must be washed before exposure to the other, for the presence of

pain, despite the fact that normal serum inhibits papain agglutination of sensitized cells (Wiener and Katz, 1951). In some circumstances[24] the agglutination of cells under

that some blood group antibodies may be found in the alpha or beta globulins. Though it is possible by suitable

use an antiserum that has been prepared against whole serum, so that sensitizing antibodies can be detected in whatever globulin fraction they may chance to occur.

The test is particularly appropriate for the diagnosis of haemolytic disease in the newborn animal, for one can determine at once whether or not the patient's cells are sensitized with antibody This not only provides the most relevant information immediately upon birth, but avoids the complication of pondering over which, if any, of the maternal antibodies is reaching the infant and doing harm; thus no detailed knowledge of the blood groups of the species under investigation is necessary for the application of the test Nor is it limited by the species, for it is as easy to prepare antisera against the globulin of pigs and horses

anti-A or anti-B in human cases of haemolytic disease

enzyme effects full agglutination of the inagglutinable class of ox cells.

The Antiglobulin Reaction

Apart from the empirical methods of modifying the menstruum or treating the cells with enzymes, there are two other means of detecting the sensitization of cells which are serologically rational. One of these is complement fixation, which is discussed later, and the other is the antiglobulin reaction of Coombs, Race and Mourant (1945 *a*, *b*). This test is based on the assumptions, first, that cells sensitized with, say, anti-D will have adsorbed particles of the antibody over the cell surface in a patchy distribution no doubt corresponding with the antigen sites; and second, that such antibody particles, being in fact particles of serum globulin, would act as though they were antigens on the cell, just as so many antigenic patches on the surface, so long as they remained firmly fixed to the cell and were not too readily dissociable. These assumptions appear to be justified, for the adsorbed globulin on the sensitized cells is easily recognized by an antiglobulin antibody, which causes the cells to agglutinate but does not, of course, affect unsensitized cells In the preparation of the antiglobulin serum, all that is necessary is the immunization of some foreign species with the serum of the species under investigation. For the diagnosis of haemolytic disease in man, it is customary to immunize rabbits or goats with human serum and to refine the resulting antiserum by appropriate adsorptions.

The question that first arises is what is the nature of the adsorbed globulin in the sensitized cells. Coombs and Mourant (1947) showed that the antiglobulin reaction on the cells sensitized with anti-D was neutralized by the addition of gamma globulin fraction but not by alpha or beta globulin, concluding that incomplete anti-D was principally composed of gamma globulin Dacie (1951) confirmed this view as far as cells sensitized with incomplete anti-D and anti-A, as well as with the adult haemolytic anaemia antibody, were concerned, but this did not apply to cells sensitized with a cold auto-antibody. However, from later work it appears

the use of antiglobulin serum on cells modified by trypsin or papain, in an attempt to increase sensitivity.

Antibody Action

Even with a mastery of all the techniques described for the *in vitro* recognition of the incomplete antibodies, one is left in doubt which properties of the antibody are being measured and with what accuracy. Serological titres are crude measures by any standard, and refinements in determining the end-point do not create any additional confidence in the method. Neither antibody titres nor measure-

[illegible]

cells *in vivo*, but sensitization may prove difficult to detect *in vitro* unless adult cells are used.

Attempts have been made to compare the sensitivity of the antiglobulin test in relation to the other methods of detecting incomplete antibodies. But the assessment that is being made is not between the efficiency of one technique and another, but merely a measurement of the potency of

and Gordon (1953) have made use of this fact in devising a method of estimating roughly the degree of sensitization of cells. They compare the titre of a standard antiglobulin

encounters sera that exhibit a prozone when used with

found in an incomplete form, none has so far been unequivocally described.

mediately of interest, the ability to pass from mother to young and destroy the cells of the latter, still eludes definition and measurement by present techniques. No single measurable property of antibody action *in vitro* can be used as an index of the *in vivo* capacities of the antibody.

also incomplete but found in the beta globulin fraction,

cance of serological findings must remain in doubt.

effect on the specific cells *in vivo*. It may be that some blood group antibodies that are not found in the gamma globulin fraction require complement for sensitization.

The haemolytic activity of human iso-antibodies is held by some to parallel the phagocytic destruction of red cells (Bonnin and Schwartz, 1954; Salmon, 1956), but Mollison *(1956 b)* has suggested that *in vitro* haemolytic activity is an index of *in vivo* haemolytic power leading to intravascular destruction of the cells. In species other than man some iso-antibodies bring about *in vitro* haemolysis. The haemolytic properties of mares' antisera do not correlate very closely with their titres by agglutination or sensitization to the anti-globulin test (Bruner, 1950; Millot and Gorius, 1950) In

though there is little doubt about their ability to cause intravascular haemolysis *in vivo*. In such studies as these the source of complement needs careful consideration, for the results might be confused if natural species antibodies against the cells under study were present in the serum used as a source of complement.

mixture of antibody molecules with differing physico-chemical attributes but with the same specificity Some authors refer to such a state of affairs as a "spectrum". The difference between the antibodies in different patients probably lies in the varying proportions of the components. That there are several components in a given sample of antiserum is suggested not only by the multiplicity of results obtained when several methods of testing for incomplete antibodies are used, but also by the many observations of serum fractionation and analysis by electrophoresis or with the aid of chemical or physical agents. The property that is most im-

mediately of interest, the ability to pass from mother to young and destroy the cells of the latter, still eludes definition and measurement by present techniques. No single measurable property of antibody action *in vitro* can be

ination. When the active antibody was anti-D or anti-Kell the incompatible cells were eliminated at a steady rate with a half period of 20-30 minutes With certain other anti-

gamma globulin fraction, caused the elimination of red cells principally in the spleen. The latter antibodies, which were also incomplete but found in the beta globulin fraction, caused sequestration of cells in the liver It may be that

cance of serological findings must remain in doubt

The haemolytic ac
by some to parallel
(Bonnin and Schwar
(1956 *b*) has suggested that *in vitro* haemolytic activity is an index of *in vivo* haemolytic power leading to intravascular destruction of the cells In species other than man some iso-antibodies bring about *in vitro* haemolysis. The haemolytic properties of mares' antisera do not correlate very closely with their titres by agglutination or sensitization to the anti-globulin test (Bruner, 1950, Millot and Gorius, 1950) In

though there is little doubt about their ability to cause intravascular haemolysis *in vivo* In such studies as these the source of complement needs careful consideration, for the results might be confused if natural species antibodies against the cells under study were present in the serum used as a source of complement.

ably lies in the varying proportions of the components. That there are several components in a given sample of antiserum is suggested not only by the multiplicity of results obtained when several methods of testing for incomplete antibodies are used, but also by the many observations of serum fractionation and analysis by electrophoresis or with the aid of chemical or physical agents. The property that is most im-

tion from the later "acquired" haematuria seen in older foals, he also laid the cause of the disease on hybridity. A little later, a most valuable contribution from Chicoli (1861)

France led to an official investigation which was reported on by Bernardin; whether Bernardin's account appeared under his own name is not clear, but his observations were given in some detail by Sanson (1863). In this account both the seriousness of the condition and the economic loss from one mule foal in ten being affected were emphasized The autopsy accounts were quite detailed, both the liver and spleen being described as enlarged, engorged and friable;

CHAPTER V

THE DISEASE IN THE HORSE AND THE MULE

Historical Aspects

[illegible]

eenth century, judging from the tone of the communications of Gellé (1803) and Texier (1807) to the newly formed agricultural society of the Deux Sèvres.[25] Gellé attributed the condition to anaemia and weakness of the mares suffering from malnutrition, and Texier in giving the full clinical description, speaks of "l'idiosyncratie bilieuse et mélancholique du baudet"

The first full account of significance is that of Carrère [illegible]

[illegible] represent the earliest useful descriptions and [illegible] condition to hy- [illegible] veterinarian may

and mentioned the possible association with neo-natal jaundice in man, believing the liver to be disordered. By the end of the century the disease had come to be considered as a haemorrhagic nephritis probably of streptococcal origin (Cadeac, 1896) From then on the possibility of microbic infection was increasingly being examined as a possible cause, and probably this view resulted in part from the wave of interest in bacteriology in France that followed the work of Pasteur.

Few important contributions seem to have been made from this time, except perhaps Powell's (1905) not entirely typical description of the disease occurring in two sibling foals in England, until 1924 In this year Sausseau reviewed the subject and concluded that the disease in mules was an haemolytic anaemia dependent in some way upon the hybrid mating, and Mossu, Giradeau and Maubaret expressed a very similar view at the same time. However, later in the same year, Donatien, Lestoquard and Sausseau [illegible]

the rats' syndrome to icterus in newborn mule foals In [illegible]

ing hybridity as a cause he *said*: "*c'est dans le sang de la* mère qu'on doit rechercher la raison de ces phénomènes, leur explication". He agreed with Chicoli that the substitution of cows' milk for suckling by the mare offered the best chance of survival.

In many of these early accounts the manifestation of haemoglobinuria had attracted more attention than had the jaundice, and Lhomme (1867) referred to the condition as "L'hématurie des jeunes muletons pendant le premier âge". Ayrault in the same year discussed the matter fully in his book on mule-breeding in Poitou, though he had published a paper on it as early as 1856 and mentioned it in his handbook of 1859 He dismissed the term "pissement de sang" under which the *disease had been known*, because haemoglobinurnia was not an invariable symptom, and substituted "l'ictère ou jaunisse des nouveaux-nés". He described the *disease thoroughly* and noticed *that on autopsy* the liver was always found to be enlarged to two or three times its normal size, surmizing that it was evidently the seat of the malady. *In discussing the disease, however, Ayrault differed from* most other authors by stating that jaundice and staining of the amniotic fluid may be seen at birth or in aborted foe*tuses* For *this reason* he held *that the disease* may *start in* intra-uterine life and could not be ascribed to any property of the milk However, he agreed that the condition must *spring from hybridity*, *for* he had *never seen a* case *in* a thoroughbred horse or donkey

Until the time of Ayrault's book observations on haemolytic disease *in newborn foals seemed generally to be lead*ing to the view that the hybrid mating caused some toxic substance to be transmitted through the mare's milk to the foal. From then on, however, the trend led gradually away from these truths In the following year Lafosse treated the disease as a renal congestion inherited from the mother, but he remarked that mule-breeders mate those mares that have borne affected mules to stallions thereafter in order to avoid the consequences of the disease Reynal *in 1874* also implicated the kidney and regarded the disease as a form of nephritis. Hartmann in 1880 was one of the first after Carrère to describe *the condition in thoroughbred horses*

[illegible]

(Cadeac, 1896) From then on the possibility of microbic infection was increasingly being examined as a possible cause, and probably this view resulted in part from the wave of interest in bacteriology in France that followed the work of Pasteur.

Few important contributions seem to have been made [illegible]

was an haemolytic anaemia dependent in some way upon the hybrid mating, and Mossu, Giradeau and Maubaret expressed a very similar view at the same time. However, later in the same year, Donatien, Lestoquard and Sausseau claimed to have identified the long-sought infective agent as *Nuttalia equi*. Consequently Sausseau in his book on the [illegible]

lytic anaemia of the newborn rat produced by injecting or giving by mouth, antisera prepared in rabbits against rat red cells Caroli and Bessis were struck by the similarity of the rats' syndrome to icterus in newborn mule foals In [illegible]

mule foal all subsequent offspring sired by donkeys are icteric, but those sired by horses [illegible] attention to the similari[illegible] to those seen in hum[illegible]

[illegible] evidence. The haemato-l[illegible] were shown to be consistent with the view that the natural course of the disease was an acute haemolytic anaemia. Serologically, Caroli and Bessis showed that the serum of mares that had borne affected mule foals had a significantly higher titre of agglutinins against donkey and mule red cells than could be found in the serum of mares bearing healthy foals. The injection of donkey blood into mares that had borne affected foals was followed by a sharp rise in agglutinin titre suggestive of a secondary immune response. Moreover, the serum of icteric foals contained agglutinins against the red cells of the donkey and mule but not against those of the horse. From this they concluded that transfusion of horse blood would be a suitable treatment for icteric mule foals

This paper was followed in the same year by two further reports. In one of these the serological findings were extended, and presented in more detail. The average serum titre of anti-donkey agglutinins in 15 mares which had borne icteric mule foals was 43·4, but that of 11 mares bearing seemingly healthy mule foals was 12 6, whereas that of 17 bearing only horse foals was no more than 0 82. No such difference was demonstrated between those three groups in examining the sera for agglutinins against the red cells of the rat or of man (Caroli and Bessis, 1947 *b*) The other report referred to the finding of an incomplete form of antibody in the serum of three mares bearing icteric foals (Bessis and Caroli, 1947).

Later in the same year Caroli and Bessis presented all this evidence in a comprehensive review and described the haematological and histological changes of their cases in some detail, the latter being illustrated by photo-micrographs (Caroli and Bessis, 1947 *c*), the same material was also published in Bessis' monograph *"La maladie hémoly-*

tique du nouveau-né" in the same year. In addition to the serological evidence previously reported, the authors showed that anti-donkey agglutinins are demonstrable in the milk of mares bearing icteric foals. The account ends with a table comparing the principal features of the disease in man, the foal and the rat. The serological evidence, though not as yet perhaps conclusive, very strongly supported the hypothesis advanced Although at this time Bessis assumed that the antibody was transferred from mare to [illegible]

Millot, 1949)

The almost contemporary study of Bruner and his colleagues in America concerned thoroughbred horses. In 1947, the year of Caroli and Bessis' reports, Dimock, Edwards and Bruner, in a review of about 200 post-mortem examinations carried out on equine foetuses and foals supposedly dead from infectious disease, noticed that several jaundiced foals yielded no pathogenic bacteria on attempts at culture Though the cause of death in these foals was not discovered they remarked that "there was a suggestion of a condition similar to erythroblastosis fetalis in human infants" In the following year they pursued this idea in a short paper describing their observations on six cases of [illegible]

cases of neo-natal icterus in thoroughbred foals at Newmarket, described by Coombs, Crowhurst, Day, Heard,

and the detailed haematogical data of three foals throughout the course of the illness.

Brion had also been working on the problem at this time. He sought to ascribe the passage of the immunizing antigen from the foetus to the mother to placental lesions caus-

Both these observations require confirmation Brion later advanced some experimental evidence in support of his hypothesis and printed some photographs of the placental lesions (Brion, 1952).

Iso-Immunization

The incid
tainly know
bidity was
nineteenth
recently (S
suggested t
naturally The incidence in horse foals, where there is less obvious hybridity, may well vary from one locality to another, depending upon the blood group distribution in the horse mating groups Cronin (1955) put the figure at 1% for thoroughbreds in the Newmarket area over a period of four years

The incidence will also depend upon whatever factors are

responsible for iso-immunization in pregnancy. Brion's hypothesis that partial separation of the placenta is responsible might lay the onus upon some endocrine disorder, as Laffolay (1949) has suggested. There is, of course, the possibility that immunization may be artificial because of the administration of vaccine containing blood group antigens incidentally. Indeed, Doll, Richards, Wallace and Bryans (1952) found that red cell agglutinins appeared in the serum of the mares receiving an equine virus abortion vaccine during pregnancy. This might be expected, for the vaccine is prepared from homogenized lung tissue from aborted foetuses and would probably contain red cell antigens. Mares already naturally immunized show a small rise of agglutinin titre against the stallion's cells after this form of vaccination, but the increase is transient. Mares not previously immunized are not usually affected, but Doll *et al.* found that in about 7% of 783 mares weak agglutinins could be detected. In a very few cases, however, they showed that the antibody was of sufficient strength to cause haemolytic disease (if the foal's cells were incompatible) particularly when the vaccine had been administered during the last three months of pregnancy. In some of the mares the antibody titres, which had risen in the early stages of pregnancy following vaccination, fell again in mid-pregnancy only to rise again just before term. At first sight this suggests that transplacental re-immunization may be found in late pregnancy in the mare. This finding was strikingly confirmed by Cronin (1955), who observed that a rise in red cell antibodies in the serum of the mare during the month before the foaling was pathognomonic of haemolytic disease in the foal; indeed, he recommended this investigation as an essential prognostic test. In Cronin's cases when the serum titre did not rise during the month before foaling, the cells of the foal were found to be compatible with those of the dam, so at present there is no conclusive evidence of the opportunity being present for re-immunization during pregnancy but no such immunization occurring.

This rise in titre towards the end of pregnancy differs from the general experience in man. However, the obstetrical histories in the two species are not dissimilar. As in

man, the mare usually bears one or more healthy foals be-
... reports of primi-
...ls have appeared
... Molay, 1951; Brion
...oll, 1948; Cronin,

though several blood group systems are concerned in the production of this disease, the selection of a stallion whose
... the immunized mare
...at no single antigen
...rominently concerned
(thus differing from the human Rh antigen D).

Immunization has been induced experimentally in this

evidence of disease in the foals One of the mares developed agglutinins in low titre, but the foal, which was suckled freely, remained healthy The fifth mare developed high titre agglutinins in the serum and in the colostrum, but the haemolytic titres were low. The foal experienced quite a severe attack of haemolytic anaemia with jaundice, but

with intravenous injections, repeated once or twice, of the red cells from about 80 ml of the blood of the donkey that had sired the mares. The antigenic dosage was thus about the same as in Bruner's experiments, though the interval between injections, about two and a half weeks, was rather

longer; the last injection to one mare was given intraperitoneally. The first mare so treated (Brion, 1950) had an agglutinin titre of 256 in the serum and 500,000 in the colostrum. The response of the foal was severe and rapid; the red cell count had fallen from 7·3 millions to 1·3 millions in eight hours, and the foal died twenty hours after birth. There is no doubt that this was a case of experimentally produced haemolytic disease of the newborn. The second mare treated in this way (Brion, 1951) had borne no foal previously. The serum agglutinin titre at birth was 1,024 and the colostrum titre 4,000. This foal, though not so severely affected as the former, sickened with the typical signs of haemolytic disease, but responded well to transfusion and separation from the mare for foster feeding. The foal, much improved, was subsequently returned to the mare on the fourth day and soon worsened and died. A third mare was immunized before mating but received no injections during pregnancy; the foal remained healthy. A rather more detailed account of a case of haemolytic disease in a newborn mule foal experimentally induced in the same way is given by Pigoury and Charney (1950, 1951).

Clinical Aspects

It is generally agreed that the foals are always healthy at birth. However, Brion (1949 *a*) claims that they are weakly and stunted, and Priouzeau (1952) asserts that in some forms of the disease the foal is jaundiced at birth or may even be stillborn and jaundiced, these views, though shared by some, are not universally held.

The signs are those of acute anaemia. The onset may be observed at any time from about six hours after birth until about the fifth day, usually the earlier the onset the more severe is the condition. Weakness and apathy are the first indications of the sickness followed by a reluctance to feed which may well be ascribed to the weakness, for if the foal is supported it may suck well. Later the lassitude becomes extreme and the animal remains lying on its side. The mucous membranes are pale and later become markedly jaundiced. Cronin observed that jaundice was found only

in those foals that either lived for some time following the onset of the illness or first showed signs of the disease some days after birth. However, in some of the cases he described, jaundice was seen at 48 hours after birth, though commonly it may take some time to develop. Unfortunately very few serum bilirubin estimations have been made on foals with haemolytic disease, but it is remarkable that in one case described by Beijers, van Loghem and van der Hart (1950) the serum bilirubin was 15 6 mgm per 100 ml. (normal said to be 1 2 mgm per 100 ml) and yet the mucous membranes were not icteric

[illegible]

rate is accelerated sometimes to as much as 180 beats per minute and the respiratory rate may rise to as much as 80 per minute, the body temperature is usually normal All these symptoms are consistent with a severe acute anaemia and death supervenes in the majority of untreated cases.

The clinical picture has been divided into three different grades by Caroli (1949 *a*), Saint Martin (1948, 1949) and Coombs *et al*. (1948), though the terminology of each of these authors is different. In the mild form (called subacute by Coombs) the onset of jaundice is late, usually about the fifth day, and the constitutional symptoms are transient These cases usually recover without treatment The acute cases are more severe and develop most of the signs about 36-48 hours after birth; there is usually no haemoglobinuria The most severe cases, of the peracute [illegible]

drome of hydrops foetalis in man, no doubt because there is little or no transfer of antibody to the foal *in utero*. Nor

longer; the last injection to one mare was given intraperitoneally. The first mare so treated (Brion, 1950) had an agglutinin titre of 256 in the serum and 500,000 in the colostrum. The response of the foal was severe and rapid; the red cell count had fallen from 7·3 millions to 1·3 millions in eight hours, and the foal died twenty hours after birth. There is no doubt that this was a case of experimentally produced haemolytic disease of the newborn The second mare treated in this way (Brion, 1951) had borne no foal previously. The serum agglutinin titre at birth was 1,024 and the colostrum titre 4,000. This foal, though not so severely affected as the former, sickened with the typical signs of haemolytic disease, but responded well to transfusion and separation from the mare for foster feeding. The foal, much improved, was subsequently returned to the mare on the fourth day and soon worsened and died. A third mare was immunized before mating but received no injections during pregnancy, the foal remained healthy. A rather more detailed account of a case of haemolytic disease in a newborn mule foal experimentally induced in the same way is given by Pigoury and Charney (1950, 1951).

Clinical Aspects

It is generally agreed that the foals are always healthy at birth However, Brion (1949 *a*) claims that they are weakly and stunted, and Priouzeau (1952) asserts that in some forms of the disease the foal is jaundiced at birth or may even be stillborn and jaundiced; these views, though shared by some, are not universally held.

The signs are those of acute anaemia The onset may be observed at any time from about six hours after birth until about the fifth day; usually the earlier the onset the more severe is the condition Weakness and apathy are the first indications of the sickness followed by a reluctance to feed which may well be ascribed to the weakness, for if the foal is supported it may suck well Later the lassitude becomes extreme and the animal remains lying on its side The mucous membranes are pale and later become markedly jaundiced. Cronin observed that jaundice was found only

(1950) the serum bilirubin was 15 6 mgm. per 100 ml. (normal said to be 1·2 mgm per 100 ml) and yet the

rate is accelerated sometimes to as much as 180 beats per minute and the respiratory rate may rise to as much as 80 per minute; the body temperature is usually normal All these symptoms are consistent with a severe acute anaemia and death supervenes in the majority of untreated cases.

The clinical picture has been divided into three different grades by Caroli (1949 *a*), Saint Martin (1948, 1949) and Coombs *et al.* (1948), though the terminology of each of these authors is different In the mild form (called subacute by Coombs) the onset of jaundice is late, usually about the fifth day, and the constitutional symptoms are transient These cases usually recover without treatment The acute cases are more severe and develop most of the signs about 36-48 hours after birth, there is usually no haemoglobinuria The most severe cases, of the peracute

are ...

... foal, this feature is not seen in the human infant with icterus gravis. The difference may be due merely to the greater ... if indeed it is much g... haemoglobin in the f... the mode of red cell ...

... in their cases having serum levels of 15·6 and 18·6 mgm. per 100 ml. (the normal being about 1·2 mgm. per 100 ml.), though haemoglobin and antibody globulin were found in the urine. However, Pigoury and Charny (1951) found bile pigments in the urine of an experimentally induced case, but the pigments were not identified and the serum bilirubin level not recorded. The urine of this case also contained albumin in a concentration of 2 grams per litre, which suggests that the kidneys may have been damaged.

Haematological Aspects

The data for haematological changes in the icteric foal are very scanty. It is well established from several reports that a quite sudden and marked fall in the red cell count follows shortly after the newborn foal has sucked. This is well illustrated by th... derived from a sing... 7·8 millions per cu. mm. at birth. Five hours later it had dropped to 6·2 millions, and 17 hours after first sucking it had fallen to 3·7 millions, at this time, however, there was no longer any antibody detectable in the milk, and so the count remaining at 3·8 millions at 36 hours reflects

the cessation of further lysis In many of the reported cases the red cell count fell in the first 36 hours to 2 millions and sometimes even lower. In the experimental pro- [illegible]

count was determined and they observed that in one or two of their cases the levels remained low for a time before recovery started.

[illegible]

those recovering after treatment the average lowest figure was about 3 8 millions, those dying despite treatment showed a fall to about 3 0 millions The correlation of the red count with the severity of the clinical condition does not seem to be absolute. In some cases the foals were severely, and indeed mortally, afflicted when the red cell [illegible]

[illegible]

tinued to fall for several days after birth, even when the antibody level of the milk is evidently low. Indeed, the varying rates of apparent haemolysis led Cronin to suggest either that weakly sensitized cells are removed very slowly or that antibody can continue to pass the foal's intestinal [illegible]

Cronin's mild cases and the slow recovery in Coombs' cases reflect the sluggish response of the foal's haemopoietic system, for Cronin saw no reticulocytes in his cases and Coombs very few; and neither of these authors, nor Bessis (1947) nor Pigoury and Charny (1951), have found many immature red cells.

The few histological studies published do not fully resolve this problem Certainly the first case of Coombs *et al.* showed significant hyperplasia of the bone marrow as their *illustrations confirm, but no such change was found in their* second and third cases, which were also very severe The absence of extramedullary erythropoiesis is also remarkable and is a feature distinguishing the changes from those seen in the newborn infant Another difference is the marked erythrophagocytosis found in the foal but present to a lesser degree in man Haemosiderosis is marked in both species The spleen is very engorged with blood and the malpighian bodies are disordered; Doll and Hull (1951) remark that the spleens are enlarged in those cases dying earlier than 72 hours after birth, but are normal or reduced in size in those living longer. In Cronin's cases the more severe degenerative changes, particularly in the liver and kidney, *were seen in those animals that survived for several* days before death

The changes in the liver remarked on by Bessis (1947) are, apart from the evidence of [illegible] haemosiderosis [illegible] of the capilla[illegible] the liver cells [illegible] diffuse degeneration and focal nec[illegible], and there was evidence of early toxic cirrhosis with bile thrombi in the canaliculi Parenchymatous degeneration of the liver was also noticed by Doll and Hull (1951)

Degenerative changes in the kidney are described by Doll and Hull In Coombs' cases the changes seen in a photo-micrograph include, in addition to some [illegible] tion, an extension of the tubular [illegible] glomerular capsule [illegible]

of haemosiderin and the possibility of haematin or bile pigment is mentioned.

Although non-specific neuronal degeneration of the brain is described there are no reports of kernicteric changes in the foal, except for a statement by Caroli (1949 *b*) that Bertrand has seen such changes; the original report of Bertrand has not been traced, however.

Serological Aspects

titres from 32 to 32,000

usually at a higher titre than those in the serum unless

The technical problems in horse blood group work have not yet enjoyed the degree of attention paid to human

cause the auto-agglutination is obscured by the pseudo-

Cronin's mild cases and the slow recovery in Coombs' cases reflect the sluggish response of the foal's haemopoietic system, for Cronin saw no reticulocytes in his cases and Coombs very few; and neither of these authors, nor Bessis (1947) nor Pigoury and Charny (1951), have found many immature red cells.

The few histological studies published do not fully resolve this problem. Certainly the first case of Coombs *et al.* showed significant hyperplasia of the bone marrow as their illustrations confirm, but no such change was found in their second and third cases, which were also very severe. The absence of extramedullary erythropoiesis is also remarkable and is a feature distinguishing the changes from those seen in the newborn infant. Another difference is the marked erythrophagocytosis found in the foal but present to a lesser degree in man Haemosiderosis is marked in both species The spleen is very engorged with blood and the malpighian bodies are disordered; Doll and Hull (1951) remark that the spleens are enlarged in those cases dying earlier than 72 hours after birth, but are normal or reduced in size in those living longer. In Cronin's cases the more severe degenerative changes, particularly in the liver and kidney, were seen in those animals that survived for several days before death.

The changes in the liver remarked on by Bessis (1947) are, apart from the evidence of erythrophagocytosis and haemosiderosis, mainly those of engorgement and dilatation of the capillaries In Coombs' series, however, damage to the liver cells was more apparent, both diffuse degeneration and focal necroses, and there was evidence of early toxic cirrhosis with bile thrombi in the canaliculi. Parenchymatous degeneration of the liver was also noticed by Doll and Hull (1951)

Degenerative changes in the kidney are described by Doll and Hull In Coombs' cases the changes seen in a photo-micrograph include, in addition to some degeneration, an extension of the tubular type of epithelium into the glomerular capsules similar to the change seen in myohaemoglobinuria in man It is also of importance that not all the pigment granules seen in the tubular epithelium were

tination; indeed, case 4 would not have been recognized serologically but for this test. Moreover, by direct agglutination cases 3 and 5 would have been recognized with only three cell samples and case 6 with only one, whereas with the antiglobulin test strong reactions were obtained with eight, seven and nine samples respectively. Cronin (1955) also used the indirect antiglobulin test regularly and found that testing the mare's serum titre twice, at about thirty days and seven days before parturition, was a helpful prognostic indication, in his experience a rising titre at that time was pathognomonic of the disease.

A simple haemolytic test has been investigated by Millot and Gorius (1950), and is regularly used by the American workers. Millot and Gorius, using mule cells, found the haemolytic titres unrelated to, and no higher than, the agglutinating titres. The experiences of Bruner *et al.* (1950), using horse cells, agreed that the haemolytic titres of antisera bear no relation to their agglutination titres but found that the haemolytic titres are often higher. Bruner *et al.* believed that the haemolytic titre bears some relationship to the severity of the disease, and they say that whereas a haemolytic titre of 100 in the mare's serum indicates a rapid onset of severe disease, one of 10 suggests that symptoms will not be manifest until the fifth or sixth day after birth

The case of Beijers, van Loghem and van der Hart (1950) is of interest in showing that antibodies may be detected in the urine of the affected foal They confirmed this observation on another foal in the following year

agglutination (rouleaux formation) normally exhibited by horse blood. The pseudo-agglutination can be abolished by washing and resuspending the cells in saline, but this process markedly weakens the auto-agglutination

The possibility that incomplete forms of antibody might occur in immunized mares has been considered, in different ways, by several workers since the demonstration of such an antibody by Bessis and Caroli in 1947. They showed that certain mares' sera had a prozone of inhibition until quite high dilutions were reached, and also that the titres of some sera were significantly higher when horse serum was substituted for saline as a diluent An albumin diluent has also been tried by Millot and Gorius (1950), who also noticed inhibition prozones, and by Coombs, Gorius and Bessis (1950) with rather better success than the report of two years earlier promised (Coombs *et al.*, 1948). The trypsin test has also been used successfully (Beijers, van Loghem and Borstel, 1951).

Some interesting observations have followed the use of the antiglobulin test, in this case a rabbit anti-horse globulin serum By this technique it was possible to demonstrate clearly that the foal's cells are not sensitized at birth but become so only after sucking, thus confirming the view that the transfer of antibody from mare to foal was by the colostrum and milk rather than across the placenta. The case of Millot and Gorius referred to on page 86 illustrates this point, for at five hours after first sucking (during which the red cell count had fallen only from 7·8 to 6·2 millions per cu mm) the antiglobulin test was negative on the mule foal's cells, but by seventeen hours after sucking the test was positive

The use of the antiglobulin test to detect incomplete antibodies in the mare's serum was first studied in detail by Coombs *et al* (1948) Their six immune sera were tested against a panel of fourteen cell samples both by simple agglutination and by the antiglobulin test in an attempt to measure the agglutinating and incomplete antibodies in the sera, employing a large panel of cell samples to make available as many antigens as possible Most of the titres were much higher by the antiglobulin test than by agglu-

Szent-Iványi and Széky).[28] These authors in their first report described four litters with a high mortality, giving a short account of the clinical and *post-mortem* findings and establishing the blood group incompatibility by agglutination tests Their later account is based on the observation of sixty litters in which the mortality was again high

The British authors (Buxton and Brooksbank, 1953 *a* and *b*) also described litters of jaundiced piglets dying during the first three days of life, and in giving a brief account of their clinical and *post-mortem* findings, ascribed the condition to blood group incompatibility They later showed that though the piglets' cells were not sensitized at birth (using the antiglobulin test) they became so after sucking, even if this was delayed for twenty-four hours (Buxton, Brooksbank and Coombs, 1955) The brains of some animals were diffusely stained with indirect-reacting bilirubin alone, though other bile pigments, including a direct-reacting bilirubin, were found in their serum Some further litters from these sows were studied by Goodwin, Hayward, Heard and Roberts (1955, 1956)

There is no certainty that the iso-immunization in these field cases can be ascribed to pregnancy causes alone As far as the British sows are concerned there is the possibility that they had become immunized by injections of crystal violet swine fever vaccine This vaccine, which has been increasingly used in Britain since 1950, is prepared from pooled pigs' blood, and its administration can provoke the formation of blood-group iso-antibodies (Goodwin, Saison and Coombs, 1955). These workers submitted a group of unvaccinated six-month-old pigs to an intensive course of immunization with the swine fever vaccine, which caused the production of red cell iso-antibodies in about half of the number studied They then examined the sera obtained from pigs in the field of which 147 had not been vaccinated and 152 had, the difference between the two groups was striking In only eight of those animals that had not been vaccinated was any blood group antibody (other than anti-A) found in the serum, and the highest titre encountered in this group, using the antiglobulin reaction, was eight. A number of these animals had farrowed, some of them

CHAPTER VI

THE DISEASE IN THE PIG

ISO-IMMUNIZATION

During the period that haemolytic disease was being studied in the horse and mule, mainly between 1947 and 1952, there was little indication that the condition might occur in pigs, although it was shown by a team of workers who were familiar with the disease in the horse, that it could be induced experimentally (Bruner, Brown, Hull and Kinkaid, 1949) They immunized three sows with the boars' cells about six weeks before they were due to farrow. The litter of one sow died within forty-two hours, and the sow was then nursed by four healthy two-day-old piglets who remained unharmed The antibody titre in the sow's milk at this time was 32, possibly too low to effect any clinical change, or it may be that the foster piglets' cells were compatible with the sow's antibody or they were too old to absorb it The litter of the second sow died within twenty-eight hours and that of the third within twelve hours Jaundice does not seem to have been a feature of the condition in these animals Nor was jaundice evident in the anaemic piglets referred to by Kershaw (1950), who suggested that the cause of their disorder might be haemolytic disease, although no serological investigations were undertaken

In 1953 two reports of haemolytic disease occurring in the field appeared, one in Hungary and the other in Britain; and these were followed by a third from America concerning two litters dying in less than twelve hours without evidence of jaundice (Doll and Brown, 1954). The Hungarian report (Szent-Iványi and Szabó, 1953) was followed in 1954 by some observations on pig blood groups and a fuller account of haemolytic disease of piglets in 1956 (Szabó,

antibodies are responsible for the disease, some more commonly than others. This is suggested by the observations that haemolytic disease may occur, though less commonly,

The possibility that iso-immunization of Essex and Wessex sows in Britain is brought about by crystal violet vac-

disease among piglets of unvaccinated sows, even though it is more common in the offspring of vaccinated ones. However, they refer elsewhere to the practice of

in one year. In Britain, however, though the widespread use of the crystal violet vaccine ensures that a substantial proportion of the breeding sows produces antibodies incompatible with all the offspring of the commonest breed of stock boar, clinically obvious haemolytic disease is infrequent.

The apparently low incidence of the disease in this

several times. Of the vaccinated group, nearly two-thirds had antibodies in the serum, and more than twenty had titres of 512 or greater Those animals that had been vaccinated two or three times had significantly higher titres than those that had been vaccinated once.

Goodwin and Saison (1956) later showed that the appearance of antibody due to swine fever vaccination was far more common in sows of the Essex or Wessex breeds than [illegible] was reactive against [illegible] compared the anti- [illegible] of which 5 had no antibody, with that of 69 Large Whites, of which 43 had no antibody. All the animals had been vaccinated once, some of them several times.

It is tempting to explain these observations by assuming that Large Whites carry a powerful blood group antigen that is not found in Essex or Wessex pigs. By analogy with man Large Whites would be regarded as rhesus positive and Essex and Wessex as rhesus negative. Such an explanation would be consistent with the higher incidence of isoimmunization among vaccinated Essex or Wessex pigs, for they would have received the vaccine containing a fair quantity of the Large White antigen to which they are presumed to be sensitive On the other hand, any antigens possessed by the Essex or Wessex breeds but not found in the Large Whites may be uncommon and not very powerful as antigens

This explanation is also consistent with the breed distribution of the disease which in Britain is predominantly confined to the offspring of Essex or Wessex sows; and sows of these breeds are usually mated with Large White boars. The reverse mating, though it would be interesting from an immunological point of view, does not occur in practice. If there were a single powerful antigen frequently or invariably found in Large Whites one must assume that it is always inherited, for there are no reports among the litters of nearly 40 immunized sows in Britain of any litter in which some piglets had sensitized cells and others had not, i.e. either every piglet in a litter has sensitized cells or none has But it may be that several blood group antigens and

be judged quite severe by human standards and yet which would pass unnoticed in the field.

The animals are healthy at birth The three significant signs that may develop in haemolytic disease are pallor,

remain active enough to suck even though it may have a profound anaemia

Jaundice is commonly seen in this condition It may not

explanation of this finding must await the further elucidation of pig blood groups.

The third observation that may contribute towards the explanation of the low incidence of haemolytic disease concerns the maintenance of the sow's antibody titre after vaccination. In four sows described by Goodwin and Saison, the high titre remained stable for about twenty months after the last vaccination. Litters produced during this period

pregnancy, returning to the former level later; a similar

They show that after experimental vaccination, even when repeated, the titre reaches its peak at the expected time and thereafter steadily declines. They have also seen sows which have borne an affected litter soon after vaccination and then the titre has steadily declined so that subsequent litters have been unaffected. They suggest that the immune response may be of two kinds, either the antibody is maintained consistently so that all litters may be affected (the

immune responses.

The Disease in the Piglet

Clinical Aspects

regardless of the severity of the disease. Then follows a brief but sharp rise in haemoglobin at about twelve to fifteen hours accompanied by a reticulocyte response. Finally, the haemoglobin resumes its fall, though here the steepness is generally more closely related to the severity of the condition. In those animals that recover the haemoglobin level may remain quite low (just above 3G. in piglet 43 of Goodwin *et al.*) for several days before rising again; a similar feature is seen in the foal.

It is easy to speculate on the meaning of these observations, though there is no experimental support for the ideas. It may be suggested that the initial fall in haemoglobin and its constant rate illustrate maximal elimination of red cells, or very nearly so; presumably in all but the mildest cases the amount of antibody so suddenly absorbed by the newborn piglet suffices to initiate haemolysis at a maximal rate. On the other hand, there is some evidence that the haemoglobin level of the normal newborn piglet may fall quite sharply under physiological conditions, and reach a level as low as 6G. within a few hours (Evans and Goodwin). The initial component of haemoglobin fall, therefore, may be in part a normal phenomenon.

The "spike", being associated with reticulocytosis and normoblastaemia, may represent an attempt to supplement the number of circulating red cells either by recruitment from some store or by increased production, or by both means. No doubt this intermission is very brief or submerged in the most severe cases, for it is fleeting enough in the three-hourly samples of moderately severe cases. It is not quite clear what the stimulus that induces this temporary remission may be. There is no extra-medullary erythropoiesis and the bone marrow is only moderately active (Evans and Goodwin), the effect of anoxia on the newborn piglets' erythropoietic system is not known, nor the influence of the products of haemolysis.

If it is supposed that the antibody is maximally absorbed by the cells just after birth and that a little later some more red cells are brought into circulation, the amount of active antibody then remaining will presumably determine the rate of haemoglobin fall in the final component, and also the

appear if the disease is very severe, for the animal may be dead from acute anaemia before jaundice has had time to develop; nor may it be found in very mild cases presumably

tion. In moderately icteric piglets the serum bilirubin level does not exceed 4 mgm. per 100 ml.; by comparison jaundice may not be evident in a piglet having a bilirubin level of 1·8 mgm. Normally there is no bilirubin in the serum of

some cases. More severely affected litters may show more marked pallor and varying degrees of jaundice, these manifestations reaching their height between about twenty-four and seventy-two hours after birth. In the most severe cases pallor and prostration develop early, haemoglobinuria is evident and death occurs during the first forty-eight hours. If the animals die from haemolytic disease they usually do so no later than the fourth day after birth.

Haematological Changes

In severe cases the haemoglobin level falls abruptly; in Doll and Brown's cases, for example, which were dead within twelve hours, the haemoglobin values of the moribund piglets lay between 2·2G. and 3·2G. and the P.C.V. between 5 and 8. The graphs in the paper of Goodwin *et al.* (1956) give some idea of the steepness of the decline in moderately affected piglets, the level in piglet 30, for example, falling from nearly 13G. to 2·2G. in twenty-eight hours.

A feature of the fall in haemoglobin observed by Goodwin *et al.* on three-hourly samples was an interruption or

generally during the first twelve hours, is quite steep and appears to show much the same rate of haemoglobin fall

The kidney suffers degeneration and necrosis sometimes affecting the entire nephron In the animals that die early, [illegible]

haemorrhages may be observed in the superficial part of [illegible]

tubules, and in the lumina [illegible]

parenchymal cells, with bile pigment deposits in the latter. No increase in extramedullary erythropoiesis is seen The spleen is commonly congested, as Doll and Brown (1954) observe No kernicteric changes were found in the brains of the animals studied by Goodwin *et al* (1956) In none of the organs studied has any increase in extra-medullary erythropoietic activity been demonstrated These histological changes were described independently by Goodwin *et al* (1956) and Szabó *et al.* (1956)

SEROLOGICAL CONSIDERATIONS

[illegible] owerful blood group [illegible] White breed of pigs [illegible] zation of Essex and [illegible]

outcome of the disease itself. A considerable excess of antibody in these circumstances would doubtless maintain the

level may represent a balance of destruction and production of red cells until the antibody is ultimately eliminated. The speed of haemolysis appears to be a determining factor throughout, and if the haemoglobin does not fall below

of prognosis

Another illustration of the importance of time as a factor in the piglets' struggle to survive is the level of the reticulocytes and normoblasts. The initial response is detected about the time of the haemoglobin "spike". In two piglets recorded by Goodwin *et al.*, the reticulocytosis at this time was 12% (number 28), 5% (number 27). The former animal, however, was undergoing the more acute haemolytic crisis and the reticulocytes had risen to 22% on the second day a little before the animal died; piglet 27, however, in whom the haemolytic process was a little less acute, exhibited a reticulocytosis of 44% at that time, and on the third day a figure of over 50% just before its death. This last animal, too, can be compared as regards normoblast and reticulocyte response with piglet 43 which recovered: the response was swifter and greater in the animal

Histological Changes

Deposits of haemosiderin and of several other pigmented derivatives of haemoglobin are widespread in most tissues and, as in the horse, erythrophagocytosis is pronounced in many organs, and particularly so in the lung. The organs showing the principal changes are the kidney and the liver.

The antibody levels in the colostrum are commonly [illegible]

[illegible]

hours after birth. It is not clear for how long the gut remains permeable to antibodies, but this time may be no shorter

[illegible]

in the pig.

offspring, must await the elucidation of pig blood groups for its confirmation or denial. Preliminary analyses of some of the antisera encountered in haemolytic disease show that they contain the antibodies of several specificities, but how many of these contribute materially to the piglets' sickness is not determined. The analysis of these sera, when complete, will establish a new basis for pig blood group studies and should also throw some light on haemolytic disease in this species.

One aspec [illegible] haemolytic [illegible] win and Co [illegible] group system is not concerned in the disease in piglets. The naturally occurring anti-A antibody may be found in high titre both in the serum, though here there are unexplained and wide fluctuations, and in the colostrum. But in the newborn piglet [illegible]

[illegible] Hence, during the critical stage when haemolytic disease might have developed, the cells do not exhibit the A antigen. Moreover, it is possible that the anti-A from the colostrum may be neutralized by the soluble A substance present in the gastric secretion (Goodwin and Coombs, 1956)

With antibodies other than anti-A Goodwin and Saison (1957) found that piglets do not usually die if the sow's serum titre is less than 512. They used the antiglobulin test, for it had previously been shown that neither agglutination nor haemolysis, whether complement from pigs, guinea-pigs, or rabbits was used, would detect anti-A antibodies satisfactorily, and that trypsin modification of the cells, [illegible]

[illegible]

units per gram These animals showed evidence of anaemia in twelve to sixteen hours followed by jaundice, rarely haemoglobinuria, and died about twenty-four hours after the injection. With doses less than 15 units per gram the animals showed anaemia and sometimes mild jaundice after twenty-four hours, and they recovered in about a week

Bessis and Freixa examined the blood and organs of a number of these animals. The number of red cells fell quite [illegible]

manifest), and by twenty-four hours it had reached 0 8 millions, two hours later the animal died The white cell count rose from 7,000 to 19,500 during the same period By contrast, in an animal receiving only about 8 units per [illegible]

during the same period A tendency to autoagglutination

CHAPTER VII

THE EXPERIMENTAL PRODUCTION OF HAEMOLYTIC DISEASE OF THE NEWBORN

Hetero-Immunization: The Guinea-Pig and Rat

The production of haemolytic anaemia in adult animals by hetero-immunization is almost as old as the science of immunity. It was attempted by Belfanti and Carbone in 1898 and has been studied since by such distinguished workers as Bordet, Ehrlich and Morgenroth, Muir and McNee and Banti. The work of Dameshek and Schwartz in 1938, in which an haemolytic anaemia was produced in guinea-pigs by the administration of a rabbit antibody against guinea-pig red cells, showed the basic features of this experimental condition in adults They found that the degree and speed of onset of the anaemia, as well as the appearance of haemoglobinuria, depended upon the dosage of hetero-antibody used They also demonstrated the condition of spherocytosis and increased fragility of the red cells which preceded the marrow response and which succeeded the rapid intravascular haemolysis.

Although their paper is the *locus classicus* for the study of experimental haemolytic anaemia in the adult animal, there has been no successful study of iso-immunization in young guinea-pigs Mynors, Heard and Coombs (1950) attempted to produce haemolytic disease in newborn guinea-pigs, but repeated inoculation of the does with the bucks' red cells failed to produce iso-antibodies. The antiglobulin [illegible]

the experiment

An account of the work of Bessis and Freixa on the induction of haemolytic anaemia in newborn rats is given

At first, four blood group antigens in dogs (A, B, C and D) were described (Christian, Ervin and Young, 1951), and these were later increased to seven by the subdivision of group A and the addition of groups E and F. The commonest and most powerful of the antigens is A. Anti-A effects equally agglutination in a saline medium and haemolysis in the presence of fresh guinea-pig serum; fresh dog

agglutinated in saline by fewer samples of anti-A and to a lower titre, though when suspended in serum or albumin they are usually slightly more reactive. Nevertheless, all samples of A′ cells are sensitized by anti-A as demonstrated by the antiglobulin test. The degree of lowered reactivity of A′ cell samples varies, and in all these respects the proper- antigen. Swisher and antibodies may be roperties of anti-B, of anti-A, for they ositively with antiglobulin sera and do not cause haemolytic disease of the newborn.

From these studies it seemed clear that the A antigen would prove most suitable for the study of transfusion reactions and haemolytic disease, accordingly A negative

and rouleaux formation was noticed together with spherocytosis and erythrophagocytosis.

Histologically, the main features observed in the enlarged livers were capillary dilatation, islands of erythropoiesis and occasional alterations in the liver cells with marked pigmentation usually giving a negative Prussian blue reaction Erythrophagocytosis was marked. The authors commented on the fibroblastic reaction seen in this organ and on the large quantity of pigment present,

haemolytic anaemia with haemoglobinuria.

An interesting feature of the experiments of Bessis and Freixa concerns the production of the same syndrome by the administration of the antiserum by mouth. Rats less than twenty days old developed a fatal jaundice and anaemia with haemoglobinuria after receiving 28 units or more per gram of antiserum by mouth; death occurred in less than twenty-four hours. Two rats receiving 21 and 14 units per

ical, haematological and pathological changes in the affected rats were similar whether they had received antiserum by mouth or intraperitoneally.

Iso-Immunization: The Dog

The work of Young and his colleagues on the dog during the years 1948 to 1952 came at a time when haemolytic disease had been seriously studied only in man and the horse. A logical approach to the study of experimental haemolytic disease, which they adopted, is first to define

born animal.

erythrophagocytosis of the specific cells but that anti-B and anti-C could not

at any time in the recipient's serum

Attention was then given to experimental haemolytic

against the canine A factor (except two, in which other factors were used) and subsequently mated to A positive sires The antibody titres slowly declined during pregnancy, and did not rise post-partum, and it is therefore reasonable to assume that no transplacental immunization took place The antibody titres in the colostrum were higher than those in the serum, but antibody was no longer detectable in the milk by three weeks after parturition The antibody was transferred to the pups solely through the colostrum and

readily on the first day of life but not thereafter An example of one of the several experiments which led to an appreciation of these points is found in litter E, which con-

former, the elimination of the donor cells was much slower, between 15 and 30% remaining at twenty-four hours, because either complement or antibody or both were temporarily exhausted by the effects of the first transfusion. Both complement and antibody fell sharply for about five hours [illegible]

that, though the antibody titre of the recipient may determine the rate of haemolysis, it may not determine its extent if complement is lacking.

In the same experiments haemoglobinaemia reached its maximum ten minutes after the first transfusion, but bilirubinaemia did not reach its peak until three or six hours afterwards. There was no such sharp rise after the second *transfusion because the rate of cell breakdown was slower,* but the liberation of haemoglobin into the blood proceeded slowly even if complement or antibody could not be detected, suggesting that the latter were being used up at the rate of their formation A transient leucopenia followed by leucocytosis was observed together with the appearance in the circulation of large numbers of nucleated red cells and some immature white cells There was no alteration in cell [illegible]

rapidly disappear from the recipient's circulation are destroyed intravascularly and that a relatively small proportion of the cells may be sequestered and destroyed extravascularly by the reticulo-endothelial system

In later experiments Swisher and Young (1954) showed that an immunized A negative dog receiving A_2 cells eliminated them slowly and exponentially by contrast with the rapid linear destruction encountered with A_1 cells They found further that a C negative immunized dog failed to shorten the survival time of transfused C cells, the same appeared to be true of the *in vivo* reactions of anti-B and anti-D. It also seemed that anti-A could promote *in vivo*

erythrophagocytosis of the specific cells but that anti-B and anti-C could not

at any time in the recipient's serum.

comprising over fifty pups. The bitches were immunized against the canine A factor (except two, in which other fac-

transferred to the pups solely through the colostrum and milk. No antibody was found in the serum of the A nega-

intestinal mucosa of the newborn pup could take place readily on the first day of life but not thereafter. An example of one of the several experiments which led to an appreciation of these points is found in litter E, which con-

sisted of twelve pups (six A positive and six A negative), which were delivered by Caesarian section. Two of each group were allowed to nurse their own bitch; the two A positive ones were severely affected and died, and the two A negative ones had quite a high titre of antibody in their serum and were clinically unaffected. Two more pups of each group were allowed to nurse another bitch which had been delivered five days previously so that the antibody content of the milk was then low. The two A positive pups that nursed the foster dam were mildly affected and recovered, while the two A negative pups had a low titre of antibody in the serum and were clinically unaffected. The remaining two pups of each group were fed on cows' milk for the first sixteen hours of life and then returned to their own dam The two A positive pups were not clinically affected (though one had a transiently positive antiglobulin reaction), and in the serum of only one of the A negative pups could any antibody be detected

Of the twenty-four pups that had been exposed to incompatible antibody nine died (and three more were killed) and yet the response of the animals was perhaps not as marked as might have been expected. The degree of anaemia was very variable and the number of nucleated red cells and reticulocytes was not striking; haemoglobinaemia was slight or absent and jaundice was mild and not common. The osmotic fragility of the cells in the affected animals was raised, however, and spherocytosis was marked in the more severe cases; there was a tendency towards [illegible]

the twelve affected pups revealed varying degrees of splenomegaly and hepatomegaly, but in some instances the liver and spleen were no larger than in unaffected litter mates Extramedullary hematopoiesis was well marked in both normal and affected pups, but the erythroid cells of the marrow, liver and spleen were less mature in the affected pups Erythrophagocytosis was a prominent finding in the sinusoids of the spleen, lymph nodes and liver of the

severely affected pups." The two litters of pups born to dams immunized against the canine C factor did not result in haemolytic disease in any of the twelve C positive pups.

Iso-Immunization: The Rabbit

[illegible] found to be comparable in titre in the serum of the newborn rabbit of the same group. Keeler and Castle (1934 *a*) re- [illegible]

showed that even when immunized during pregnancy, the doe's antibody titre declined during the last part of this period and rose again after parturition This decrease in titre also occurred, though to a lesser extent, with compatible offspring, though when the post-partum rise took place in these circumstances is not clear These observations were similar to those made on the human subject at this time, and any discrepancies may be partly due to an unrefined serological technique, titre in these experiments, for example, refers merely to the strength of the agglutination of red cells by neat antiserum.

In 1947, Nachtsheim and Klein observed a condition in certain newborn rabbits in which both hydrops and erythroblastosis were prominent features, together with marked

haemopoiesis in the liver and spleen; Dahr (1947) undertook some serological investigations which were inconclusive. This work, however, was not continued beyond a preliminary stage, and some of Nachtsheim's rabbits were sent to Cambridge without unfortunately their producing any further examples of the condition.

An attempt was made by Heard, Hinde and Mynors (1949) to produce haemolytic disease of the newborn experimentally in rabbits. Nineteen does were immunized against the cells of the buck to which they were subsequently mated, and this procedure was repeated for several pregnancies Nine of the does produced antibodies against the buck's cells, in three of which the antibody was of the incomplete variety (detectable by the antiglobulin test), and in six it was an agglutinating type, though in three of these some incomplete antibody co-existed These authors also noticed, as Keeler and Castle ...

l

i

s

th ... of compatible offspring ... positive antiglobulin test; quite clear why there were no sensitized cells among the incompatible offspring of the other five does; in one case the maternal antibody titre seemed high enough by comparison

All the young ... fectly hea ... their litte ... cords wit ... experience of Keeler and C ... this certainly ac- any marked d ... detected by h ... of some of th ... creased haem ...

... roid-myeloid pre- ... haemoglobin and ... siderosis and ... showed erythrophagocytosis and evidence of red cell regeneration on examining splenic impression smears But these findings are not conclusive in themselves, and it is to be noted that in this investigation the kidneys, suprarenals,

bone marrow and brain were apparently normal on histological examination of all the animals under study.

A more successful attempt to produce haemolytic disease experimentally in newborn rabbits has been reported by Kellner and Hedal (1952, 1953). These authors start- as

... were virgin at the time of mating, and it is not stated whether the five that responded by producing antibody had previously borne litters. This may be important in considering the ...

only on ...

nancy, ...

partum. ...

seem to ...

pregnancies

Both agglutinating and incomplete antibodies appear to pass freely from the maternal to the foetal circulation in this species, for the titre of each in the sera of newborn rabbits against incompatible cells was the same as that in the doe's serum, and the same was true when the doe had received iso-antibody intravenously. Nearly all the incompatible offspring had sensitized cells as exhibited by the antiglobulin test, but the failure of one or two of such animals to show a positive test needs explanation. These authors also noticed that the antibody titre ...

the mammary gland when there appears to be no difficulty

creased extramedullary erythropoiesis was a constant finding.

Anderson (1956) also succeeded in inducing haemolytic

neo-natal deaths, and Anderson suggested that if death occurs early enough *in utero* the foetus may be resorbed. Oedema was the principal clinical feature, and the general appearance was strikingly similar to hydrops foetalis in

Haemoglobinuria was not seen. It is interesting that there was a considerable variation in severity among litter mates.

The haematological findings in these cases were consistent with a moderately acute haemolytic anaemia. The

erythropoietic response in the rabbit. Spherocytosis was also common, but

organs.

low antibody titres in the cases of the former workers, the latter workers

distribution in the various mating groups may partly account for them

In 1957 Morris communicated to the British Society for Immunology the results of some experiments on hetero-immunization of mice, by injecting or feeding anti-sera prepared in rabbits. In young mice anaemia was accompanied by pallor and death ensued quickly following large doses of anti-sera; smaller doses provoked jaundice and bilirubinuria, and very small doses haemoglobinuria *after* twelve hours. Adult rats regularly evinced an earlier haemoglobinuria before six hours

Complement activity was detected in adult mouse (and young rat) sera, thus accounting for

CHAPTER VIII

COMPARATIVE ASPECTS OF HAEMOLYTIC DISEASE OF THE NEWBORN

Iso-Immunization

Of all the problems in haemolytic disease of the newborn, the cause and mechanism of iso-immunization is, perhaps, the most intractable. In man there is no doubt that immunization may result from the circumstances of pregnancy alone, though in clinical practice the consequences of artificial immunization by incompatible blood transfusion needs constantly to be borne in mind. When immunization occurs in man, the offending antigen is D in over 90% of

that gives rise to it, that is only about one in twenty of the

the whole explanation

One suggestion that has recently been advanced is that normally foetal cells do not gain access to the maternal circulation and hence have no opportunity of evoking antibodies, but that in some cases a retro-placental haemorrhage may cause some foetal red cells to enter the maternal uterine sinuses so that immunization could result. Such an

turition, though this also may occur in rabbits artificially immunized during pregnancy (Keeler and Castle, 1933). Moreover, it is also consistent with the view that the antigenic stimulus is brought about by intact red cells. This last point has been much in doubt, mainly because it has been difficult to visualize whole red cells traversing the placental barriers, particularly in the horse. But this difficulty being overcome by the concept of placental haemorrhage, it becomes more convenient to think of the antigen in terms of the intact red cells Moreover, the rapid elimination from the mother of foetal cells incompatible for A and B as an explanation for the lower incidence of haemolytic disease due to anti-D in heterospecific matings, is valid only if it is held that red cells enter the maternal circulation intact. The final point in favour of the hypothesis that placental haemorrhage may lead to immunization of the mother is the demonstration that foetal red cells can in fact be found in the maternal circulation in certain circumstances (Chown, 1954 *a* and *b*)

One's acceptance of this explanation is coloured by two thoughts. First, that though the sporadic appearance of iso-immunization is thus accounted for, its persistence in a mother, once it is established, remains to be explained It is not easy to assume that a constitutional defect of physiological collaboration between mother and child becomes established in some families and not in others, unless it be due to an endocrine disturbance as Laffolay (1949) has suggested for the horse But unless one conceives of a mechanism by which ensuing pregnancies reinforce the immunization, which has not so far been achieved, it becomes necessary to consider whether the maternal immune response could be maintained or even augmented throughout the child-bearing period in the absence of further antigenic stimulus, as Goodwin and Saison (1957) postulate for the pig, and this is difficult to reconcile with the common observation that with successive children the severity of the condition worsens Neither of these views is without objection, and there may be an intermediate situation in supposing that in some families placental haemorrhage is, for some reason, not uncommon, and in these increasing severity

CHAPTER VIII

COMPARATIVE ASPECTS OF HAEMOLYTIC DISEASE OF THE NEWBORN

Iso-Immunization

Of all the problems in haemolytic disease of the newborn, the cause and mechanism of iso-immunization is, perhaps, the most intractable In man there is no doubt that immunization may result from the circumstances of pregnancy alone, though in clinical practice the consequences of artificial immunization by incompatible blood transfusion needs constantly to be borne in mind When immunization occurs in man, the offending antigen is D in over 90% of the cases, if the ABO groups are excepted. This could be accounted for if it were shown that the D antigen were about ten times more effective in evoking an antibody response than other antigens; but this remains to be demonstrated Even so, the incidence of iso-immunization is about twenty times less frequent than that of the incompatability that gives rise to it, that is, only about one in twenty of the mothers at risk develop antibodies. The reason for the sporadic appearance of iso-immunization has been a subject of considerable speculation It is unlikely that a variable sensitivity among different subjects to the antigenic stimulus is the whole explanation.

One suggestion that has recently been advanced is that normally foetal cells do not gain access to the maternal circulation and hence have no opportunity of evoking antibodies, but that in some cases a retro-placental haemorrhage may cause some foetal red cells to enter the maternal uterine sinuses so that immunization could result. Such an explanation is consistent with the observation that first-born children are usually unaffected, and with the finding that iso-antibody titres commonly rise sharply soon after par-

red cells into the maternal circulation The problem here is to determine why iso-immunization does not occur more frequently, and why its incidence is mainly confined to the offspring of group O mothers

The family history in cases of haemolytic disease due to anti-A or anti-B is rather different from that due to anti-D, as might be expected The first-born may be affected if anti-A or anti-B is concerned, and there does not appear to be any general tendency for later children to be more severely affected than their elder siblings The disease may occur in both secretor and non-secretor infants, but one would not expect a non-secretor infant to be the first in a family to be affected, unless immunization had been brought about previously by incompatible transfusion or other means unconnected with pregnancy such as the heterogenetic stimuli of various vaccines incriminated by Crawford, Cutbush, Falconer and Mollison (1952) At this stage some evidence would be most welcome bearing on why the infant's soluble blood group substance in some cases immunizes the mother but in others does not The influence of immunological tolerance on the incidence of immunization against A, B or D is not yet determined; Owen *et al* (1954) holding that some support for this theory may be found in the relevant data, but Booth *et al* (1953) finding no such relationship

In the horse, it may be that the equine abortus vaccine used in America initiates iso-immunization against blood group factors, but it seems likely that this can occur as a result of pregnancy alone The difference in incidence of haemolytic disease in mules (about 8%) and horses (about 1% in some areas) may be due to the greater antigenic difference between donkey red cells and certain horse blood groups for the mare Until the distribution of horse blood groups is clarified it will be difficult to assess what proportion of mares at risk are in fact immunized The cause of immunization in this species remains substantially unexplained

There is no certain evidence to suggest that iso-immunization occurs naturally in the pig It seems likely that the crystal violet vaccine accounts for most, if not all, of the

is the rule, but in others the initial immunization alone is responsible for the disease, and that later children are but mildly affected

progressively

only (Davies,

placental haemor-

gs to mind if the placental haemorrhage theory is accepted, is how far it applies to the horse *In this species iso-immunization also occurs naturally with an incidence not so very different from that in man, and in this species, too, the first-born commonly, though not invariably, escapes, and successive offspring after the first icteric foal are usually affected. Can it be supposed that in two such different species and with such different placentation, a similar placental lesion occurs with similar incidence? And if it does, would the retro-placental blood have such easy access to the uterine vessels in the mare? If both these concepts proved true one might wonder why other species, too, might not be more susceptible to iso-immunization than has so far been shown. It would also be curious that the rise in maternal titre late in pregnancy, said to indicate an incompatible foetus, would not be found in man if the mechanism is similar in the two species*

cause the circulations are separate. But the infant's protection against haemolytic disease due to anti-A or anti-B is of an entirely different nature, for these antibodies in their natural form are normally present in the mother's circulation even if the foetal cells are incompatible with them; and further, iso-immunization against A and B is not unusual and probably occurs more frequently than haemolytic disease (Levine, 1955) Immunization against these factors is probably effected by the soluble blood group substances and so it is unnecessary, in this case, to postulate retro-placental haemorrhage or other means of passage of intact

would effect this is placental haemorrhage. The cause of the placental haemorrhage is unknown None of these theories will account for the maintenance of immunization once established. It is not impossible that the original stimulus may result in a permanently immune state even in the absence of the antigen, this suggestion has been made with respect to the pig (Goodwin and Saison, 1957), and persistence of maternal antibodies has been reported in man (Wiener, Nappi and Gordon, 1951)

Iso-immunization in the horse is less clearly associated with parturition, for haemolytic disease may occur in first pregnancies and there is some evidence that titre changes during pregnancy may be influenced by the foetus Apart from artificial vaccination which may immunize the horse, the evidence in this species points, rather surprisingly, to some intra-uterine cause of an entirely unknown character The possibility of transplacental immunization cannot be ruled out entirely in this species for, though the mature placenta is epitheliochorial, the maternal-foetal relationship may be much more intimate earlier in pregnancy. The suggestion that premature separation of the placenta may be concerned will need confirmation In the pig and rabbit, and possibly in other species too, iso-immunization may possibly occur naturally, but it is evidently so very rare that no information about its mechanism is available

Passage of Antibody to the Young

Once iso-immunization is established in the mother and the antibodies present in sufficient strength, the appearance of haemolytic disease depends upon the ability of the mother to transfer the antibodies to the young animal Such a mechanism exists no doubt to provide the infant with a suitable protection against infectious disease until it is mature enough to manufacture antibody for its own needs This matter has been the subject of many investigations since the classical studies of Ehrlich in 1892

Ehrlich, working with mice and subsequently with goats, first demonstrated the passage of antibody from mother to young, showing by wet-nurse experiments that the passive

cases of haemolytic disease in Britain, and possibly those reported from Hungary. The disease in newborn piglets is rarely described in other countries[23], but this is little indication of the incidence of iso-immunization considering the commonly unobtrusive character of the condition in this species. Nor is it very likely that dogs or cats experience haemolytic disease or iso-immunization to any significant extent. In rabbits the experience of Heard *et al.* (1949) and Anderson (1955) suggests that iso-immunization is very difficult to achieve in this species even in animals that had previously had many pregnancies ... of iso-imm ...

t ... r ... condition more frequently in g ... laboratory stock.

The available information does not at present point towards one cause of iso-immunization applying to all species and all antigens In man immunization may result ... artificial causes such as ... in the case of A and B, th ... genetic stimuli It may ... causes connect ... found th ... have not ... tolerance to the relevant antigens during their own intra-uterine life; but such evidence will need to provide suitable comparisons between the ABO and Rh groups in this respect. On the other hand, several observations at present point to some feature of parturition itself being primarily responsible. In the ABO groups the critical factor seems to be the access of the soluble blood group substance to the mother, and mothers of group O seem to be particularly liable, a point that has not yet been satisfactorily explained In the Rh group it is the red cell that apparently carries ... mother's ...

... most mobile a ... circumstance in parturition that

maternal vessels into the uterine lumen is across the yolk-sac splanchnopleur to enter the vitelline vessels and thence to the foetus. By comparison the rat, which has placentation similar in principle to that of the rabbit, though differing in detail, adopts a materially different form of passive immunity. Only a modest proportion of antibody reaches

1927).

the route already mentioned, antibodies pass from the uterine lumen into the amniotic fluid, some of which may be swallowed by the foetus and so enter the stomach

1949)

(1951) found that from the twenty-fourth day of gestation in rabbits, homologous antibodies (i.e. those contained in

immunity was reinforced by sucking. Many similar observations, made around the turn of the century, were reviewed by Famulener (1912), who was himself the first clearly to show that, in goats, the antibodies passed not through the placenta but in the colostrum to the kid. Observations on the same matter, but from a different angle, were made by Howe (1921), who showed that the euglobulin fraction is missing from the plasma proteins of newborn calves until after suckling. In 1922 Little and Orcutt and Smith and Little demonstrated the colostral transfer of immunity against *Bact. coli* septicaemia and *Br. abortus* infection in calves, and Mason, Dalling and Gordon (1930) published similar work concerning lambs All this work (except the last) was extensively reviewed by Ratner, Jackson and Gruehl (1927), who suggested that the mode of transfer of antibodies from mother to young in the various species could be correlated with the structure of the placenta They suggested that in species with the greatest number of placental layers interposed between the foetal and maternal circulations (epithelio-chorial and syndesmo-chorial placentation) these barriers were impassable to antibodies which

pass relatively freely across the placenta and colostral transfer of immunity would be unimportant This theory was further elaborated by Schneider and Szathmary (1939), and summarized in a table given by McGirr (1947)

More recent work, however, has shown this view to be unreliable, for though epithelio-chorial and syndesmo-chorial placentas seem to be impermeable to antibodies, the

(Brambell, Hemmings and Henderson, 1951) The route adopted by the antibodies after their secretion from the

the common finding that antibody in the newborn appears often to be concentrated to a higher level than that in the mother (Liebling and Schmitz, 1943, Barr, Glenny and Randall, 1949, Bryce and Burnet, 1932; Murray, Calman and Lepine, 1950, Murray and Calman, 1953), but Hartley (1951) points out that this observation may be due to a higher rate of antibody breakdown or excretion in the mother The concentration of antibody in the infant may be genuine, however, for though the total plasma globulin of the newborn infant is low compared with the mother, the gamma globulin fraction is relatively rich (Longsworth, Curtis and Pembroke, 1945)

The nature of the permeability of the relevant membrane separating the plasma proteins of mother and infant is of great interest in relation to the properties of the blood group antibodies The selectivity is not acquired until the gestation is advanced to a certain age, but when mature enough, the endodermal cells are endowed with power to discriminate not only between homologous and heterologous globulins, but between homologous antibodies of the same specificity which probably could not be distinguished by any laboratory means Hartley (1951) has shown a discrimination just as subtle effected in the guinea-pig He administered diphtheria antitoxin, made in guinea-pigs and horses, to pregnant guinea-pigs and measured its concentration in the serum of mothers and young after birth He found that the homologous (guinea-pig) antitoxin passed freely and abundantly to the foetus whereas the heterologous (horse) antitoxin reached the foetus in far lower concentration He then showed that "digestion" of the antitoxin, even of the homologous antitoxin, with pepsin before it was administered resulted in none of the treated antitoxin reaching the foetus Peptic digestion does not alter the *in vitro* neutralizing property of the antitoxin, and as it probably splits the molecule into two parts it may be inferred that molecular size is not a significant factor in membrane permeability for gamma globulin Hartley's explanation for this effect, and for the fact that pepsin-treated antitoxin does not passively sensitize guinea-pig tissues to anaphylaxis (as untreated antitoxin doses), depends upon the antitoxin molecule being

selective property did not operate as early as the twentieth day of gestation, and was found not to depend on the size of the antibody molecules. In an attempt to identify which layer, of the many traversed by the antibodies in this species, was responsible for the selection, Brambell found that the entodermal surface of the yolk-sac was the only membrane with this property, no discrimination being exerted by the amnion, chorion or bilaminar omphalopleur. The chick resembles the rabbit in absorbing antibody, not through the gut but through the yolk-sac wall, which also exerts a selective effect (Brierley and Hemmings, 1956) In the rat the selective membrane is the gut wall during the first twenty days after birth. In all these instances the dis-

the H and O typhoid agglutinins, and Adamson, Lofgren anti-staphylolysin compared with *Bact coli* antibodies And there is abundant evidence of selection of blood group antibodies such as the readier passage of incomplete anti-D compared with its agglutinating form, the immune anti-A more readily than the natural agglutinin, and the variants of anti-M (Jacobowicz and Bryce, 1951) These are even finer discriminations than the experimental ones, for differences among homologous antibodies are being recognized If this mechanism operates at the allanto-chorionic placenta it would differ from that in other species examined in being effected by a membrane that is not an entodermal surface. On the other hand, if Brambell's suggestion is correct the antibodies would reach the amniotic fluid via the chorion laeve and, possibly, become concentrated in the

rat, in which a small and possibly unimportant part of the immunity is transferred *in utero*, but the principal route is via the colostrum. In this species the property of selective permeability is exhibited by the gut (Halliday, 1955 *a*). It is remarkable that the intestinal permeability of the newborn rat lasts for eighteen to twenty-one days after birth (Bessis, 1947; Halliday, 1956), which is a much longer period than applies in ungulates and must represent a substantial proportion of the life of the rat. Halliday remarks that it is at this age that the young rat normally starts taking solid food, but the relationship does not apply similarly to foals and piglets. The absorption of antibody from the gut in rats is swift, being detectable as early as half an hour after feeding and maximal by three hours.

The other group comprises those animals, like the horse and pig, in which no immunity is transferred *in utero* and all antibodies pass in the colostrum. There is insufficient evidence to determine where, if at all, any selectivity is demonstrable. It could possibly be in the mamma, though little is known about the means of concentrating antibody in this organ. Campbell, Humphrey and Work (1954) suggest that in the rabbit the antibodies are transferred simply and directly from the plasma, and Timmerman (1931) found that in man, though the typhoid H agglutinins pass freely to the foetus, the O agglutinins were unable to do so and were concentrated in the colostrum, but in ungulates, entirely different considerations apply. Some concentration of antibody in the colostrum is common in these species, but not invariable, and it is not certain that all antibodies are concentrated to the same degree. Goodwin and Saison (1957) observed that the severity of haemolytic disease in piglets was not related directly to the degree of antibody concentration in the colostrum, after suckling the antibody titre in the milk fell during the first day or so to a level similar to that in serum.

Since the observations of Howe in 1921 that euglobulin was absent from the serum of newborn calves until after sucking, it has been shown that gamma globulin is not found in the serum of newborn calves (Jameson, Alvarez-Tostado and Sortor, 1942) or foals (Polson, 1943, Hansen and Phil-

attached to or entering the membrane cells before reaching the foetus.

known. It is, however, of great importance, for the property of some blood group antibodies to pass to the foetus is not shared by all, and it is essential to the development of haemolytic disease; moreover, this property is not always

Hemmings (1954). It may be thought of as a form of secretion; and placental secretion was hinted at as early as 1907 by Lochead and 1909 b
invoke any theory tha
cules enter the interior
side It is simpler to assume that the molecule is passed between the cells but in very intimate contact with the cell surface. Only by extremely close association with the cell

this property that is of particular interest in the evolution of haemolytic disease

Those animals that transfer their passive immunity by way of the colostrum may, in the present state of knowledge, be divided into two groups. There are those, like the

these species, some of them may not become entirely accessible to antibody in the early stages. The A antigen, for example, does not always appear to be fully reactive at birth, so that A_1 children may at first be easily mistaken for A_2. It is possible that this is solely an *in vitro* characteristic and that the cells suffer fully from antibody action *in vivo*. On the other hand, some of the evidence is consistent with the A antigen being in several cases rather less reactive *in vivo* with the specific antibody. Indeed, this factor probably forms part of the infant's defences against haemolytic disease due to anti-A. Perhaps these defences could be reconstructed thus: in the first place there is normally no iso-immunization, so that the situation worsens once the unexplained episode that occurs during parturition allows the soluble substance to immunize the mother. Secondly, the passage of antibody from mother to foetus is not always very free (though some invariably passes), but possibly "immune" or recently stimulated antibody is more readily admitted to the foetus; again the risk is small unless there is iso-immunization. Thirdly, such antibody as succeeds in reaching the foetus may be neutralized by the A antigen in the tissue cells and secretions. Therefore if the antigen is a little slow in revealing itself on the red cell's surface, the antibody may be preferentially absorbed by tissues and secretions, thus protecting the red cells This may partly explain why the symptoms are generally milder in the syndrome resulting from anti-A or anti-B No such rings of defences are discoverable against the Rh-induced syndrome, the infant seems to rely exclusively on the physical barriers, so that once these are breached, unless the mother is in [illegible]

that the placenta might do so

In man and in the rabbit, therefore, the maternal antibody may flood the circulation of the foetus and presumably becomes attached to the red cells. Even if the foetus is able to metabolize and excrete some of the antibody, more

lips, 1947), though the other fractions are present, albeit in proportions different from those in the adult. Jameson *et al.* remark that though the globulin fractions are quite distinctive by electrophoresis yet the absolute mobilities are faster than would be expected with adult ox sera, and they apparently observed a similar phenomenon when studying the sera of young rats. The new component appearing in the serum of newborn calves and lambs after sucking had the same mobility as the "T" component of ox globulin, and [illegible]

antibodies may be absorbed very swiftly and flood the circulation in a few hours, at any rate in the pig (*Goodwin et al.*, 1956) Some degree of permeability is maintained for about thirty hours but, except for the report of Cronin and Howard (1955) concerning a foal, there is little evidence that the gut remains significantly permeable to protein in these species after the second day of life.

The Effect of the Antibody

Once the membranes separating the circulations of [illegible]

The replacement of lost red cells is, no doubt, accomplished by the stimulus of anoxia In both man and the

Walker, 1955), there is no reason to suppose that these

anti-D. If this change predisposes to phagocytosis of the

is available from the mother so that an excess is usually present. There is little doubt that the infant's red cells can be destroyed in th[illegible]

[illegible]

developed so early in intra-uterine life. Moreover, much of the space occupied, in the adult, by the reticulo-endothelial system is, in the infant, given over to haemopoiesis. However, it is clear that red cells are destroyed.

The loss of red cells *in utero* in these species give rise to two sets of consequences: the disposal of red cell products and the replacement of red cells. The infant does not seem

[illegible]

claims tl [illegible]
placenta [illegible]
women [illegible] to abnormal channels so that, perhaps,

[illegible]

an intra-uterine condition This may well be so, but [illegible] foetal changes may not become irreversible until after birth It is also true to say that the changes in kernicterus could be accounted for entirely by a series of post-natal circum-

[illegible]

kernicterus from the rabbit, for Anderson (1956) believes that the newborn rabbit is able to excrete into the gut even [illegible] no-
[illegible] ely
[illegible] t a
[illegible] ile

upon the nature of the antibody. Some antibodies that are detectable *in vitro* by agglutination do not shorten the survival time of the foetal cells, e g canine anti-B, anti-C and anti-D; others fail to do so because the antigen has not developed in the young animal, e g [illegible]

[illegible]

is merely a question of degree or whether the power to destroy red cells *in vivo* is a specific property of some antibodies

The immune destruction of red cells seems to follow one of three courses. The first of these is intravascular haemolysis and is characteristic of the disease in the horse, dog and pig When the process is acute there is marked haemoglobinuria but there is insufficient time for jaundice to develop; in more slowly progressive cases haemoglobin is broken down rather than excreted and jaundice follows. The role of complement in these acute haemolytic episodes is insufficiently understood It is certainly required for such haemolysis in the adult dog, but its requirement or availability in the newborn in any species is not known It is remarkable that in the dog and pig, homologous fresh serum does not effect lysis by iso-antibodies *in vitro* and some complement component, perhaps C'3, of guinea-pig serum, is required Erythrophagocytosis is a prominent feature of the disease in these three species, and this suggests that red cells may also be destroyed by means other than simple lysis within the circulation. In man intravascular haemolysis may well occur only when anti-A or anti-B is the cause, and with these antibodies spherocytosis is common, a [illegible]

[illegible]

dence concerning the origin of damage to the liver or the kidneys in this disease, and since neither the antibody nor bile pigments can be certainly incriminated in all species, some rather less specific agent such as anoxia must be blamed. This must also be held, at present, to account for the immaturity of tissue development in human haemolytic disease commented on by Morison (1952). Why labour should often start prematurely in the human species with this condition is not known.

In turning to the post-natal development of this condition a wider range of species may be included and the circumstances are generally different from those that apply *in utero* The period during which the antibody can act is circumscribed. In the human infant the natural rate of … estimated as having … b), and … y be ex- … Lenke, … t in the infant at birth will thus determine how long red cell destruction will co… enter the ci… line, Rober… hours after … forty hours, possibly in decreasing amounts towards the end of that period On account of these features and also because of the peculiar circumstances of kernicterus in man, the events of the first forty-eight hours of life determine the

The Manifestation of Haemolytic Disease in Different Species

The effect of haemolytic anaemia in the neo-natal period varies in the different species. In man the anaemia may be so severe that the foetus succumbs *in utero* or shortly after birth. With severe anaemia the principal risk in the new-born infant appears to be that of heart failure, sometimes complicated by the precarious health resulting from premature delivery which is not uncommon in this condition. If the neo-natal anaemia is survived the infant may become vulnerable again a week or two later on account of a secondary anaemia developing, particularly in premature infants, due to the rather sluggish response of the bone marrow to anoxia not keeping pace with the child's rapid growth in size and hence in circulatory volume. The erythropoietic response in the rabbit appears to be more lively, so that if the neo-natal period is survived the animal recovers

In the horse and pig the anaemia may develop very acutely in the first twelve to twenty-four hours so that death occurs before the marrow can respond. In these species, too, erythropoiesis is not quickly stimulated so that a prolonged [illegible]

[illegible]

tion by its inability during the first week of life to convert body protein to nutritional use when its sugar reserves are [illegible]

The other two forms of red cell elimination have been described in man by Mollison (1956 *b*). In one of these,

specific cells intravascularly with the aid of complement. This linear splenic destruction of cells is presumably the usual mechanism in human haemolytic disease other than that due to anti-A or anti-B, and is consistent with the absence of haemoglobinuria The reticulo-endothelial activity is, however, accomplished with little histological evidence

for sensitization *in vitro*, and it is possible that the initially rapid destruction occurs when complement is abundant and that the later slow elimination reflects an exhaustion of complement, the activity then being limited by the rate of complement replacement. An exponential form of elimination has also been shown with canine anti-A on A_2 cells, the effect thus differing from that on A_1 cells

There is little in this evidence that conforms with the views of Bonnin and Schwartz (1956) and Salmon (1956) that antibodies that promote lysis also predispose to erythrophagocytosis, complement being favourable but not essential to these reactions, nor with the view of Wasastjerna (1953) that the same change promotes spherocytosis and

CHAPTER IX

TREATMENT

In a comparative study it becomes evident that the thera- [illegible]

[illegible] nts cannot remain un- [illegible] reatment in man must [illegible] er species. [illegible] o useful preventa- [illegible] can modify the [illegible] *mpts to inhibit or* [illegible] istration of Corti-sone or ACTH have not [illegible] ly successful; nor can one expect much from this approach to the problem Likewise the administration of the antigen during pregnancy to neutralize or "desensitize" the antibody's effects on the foetus has also proved unavailing; *this step would*

plete until after birth. If the site of dental hypoplasia that is found in this condition is an index of the age at which the metabolic disturbance occurs, the evidence points to the first two or three days of extra-uterine life (Forrester and Miller, 1955). It is not certain whether the peculiarity of human neo-natal development that renders the species liable to a kernicterus is a specific liver immaturity or a particular form of bile pigment metabolism. The consequence is to render haemolytic disease a more toxic and possibly more morbid condition in man than is usual in other species

[illegible] man and the horse has so ancient [illegible] the [illegible] It is [illegible]

blood groups In man, one must supp[illegible] haemolytic disease operates against heterozygotes so that a gradual loss from the community of the rarer gene might be expected. This would apply to the d gene of the Rh system, and the A and B genes in populations having a fairly high incidence of O. If this does indeed happen, and its rate would obviously be extremely slow, it is still possible that the effect may be compensated for by other genetic means.

CHAPTER IX

TREATMENT

In a comparative study it becomes evident that the therapeutic approach is conditioned as much by economic considerations as by the physiology and pathology of the [illegible]

differ fundamentally from that in other species

In man the disease starts *in utero*, but no useful preventative measure has been established that can modify the disease process during pregnancy. The attempts to inhibit or [illegible]

on the foetus has also proved unavailing, this step would presumably so immunize the mother that subsequent infants might be very gravely affected

It seems that only two steps can be taken during pregnancy The first of these is to establish whether or not the mother is immunized As a corollary to this the father's genotype may be determined Repeated examination of the [illegible]

cover antibody if it is there, and its presence is most likely towards the end of pregnancy. The finding of antibody enables the physician to persuade the mother to have her baby in hospital where facilities for diagnosis and prompt treatment are available.

this procedure is that, as the infant is continuously under the harmful influence of antibody *in utero*, the sooner it is

of parturition and treatment. Indeed, the uterine environment, to which the infant becomes surprisingly well adapted, is not so very

of its bile pigments,

probably better adapt

poses the infant to a number of risks. In the first place the

and bile pigment concentration; early induction ensures prematurity, and the bile pigments which the infant can excrete placentally *in utero* will rapidly accumulate after

the infant is better off with the large numbers of red cells which intra-uterine anoxia imposes together with a continuous supply of antibody, or, as after birth, with a limited but still substantial amount of antibody but no very satisfactory way of disposing of red cells and their products.

Statistically it has been shown by Allen, Diamond and Vaughan (1950) and by Mollison and Walker (1952) that no

benefit accrues from premature induction of labour. On the other hand, it is argued by Davies, Gerrard and Waterhouse (1953) and by Watson, Cross and Hatchuel (1954) that in exceptional circumstances premature induction may be called for. An example of the sort of case they have in mind is *illustrated by the latter authors*; a mother having failed on five previous occasions to bring pregnancy to term declared that she could detect a weakening of foetal movements at the thirty-third week of her sixth pregnancy similar to those preceding the intra-uterine death of some of her former offspring. She was delivered by Caesarian section and the infant received prompt treatment and appeared to survive in a healthy state; the seventh pregnancy was similar. If, therefore, the infant whose birth is induced before term is able to survive the risks of prematurity, the risk of kernicterus can presumably be greatly minimized by exchange transfusion (see below) to remove the early accumulation of bile pigments. This view is strongly advocated by Kelsall and Vos (1955), who rarely see kernicterus with this treatment. However, such premature infants may need several days for the liver to mature and a later accumulation of bile pigments might be expected even if there is no excessive red cell destruction as in "normal" premature infants (Lathe, 1955). This being so, the possibility of a second exchange transfusion being necessary on occasion calls for close supervision of the treated baby. The problem thus becomes one of estimating how far the infant can meet the general perils of prematurity compared with the risks of its uterine environment. Kelsall and Vos (1952) believe that they can assess the latter by ante-natal changes in antibody titre using the antiglobulin test. This is not easy to justify *by serological theory, but their clinical results are good.* The decision to induce labour prematurely, which can be taken very seldom, must depend upon a close study of the individual case[29].

The treatment of this disease in man is, however, principally directed towards ameliorating the condition as soon as possible after birth. The most valuable procedure is that of exchange transfusion. This is an alternating withdrawal of small volumes of infant's blood and administering of

small volumes of warmed compatible rhesus negative blood [illegible]

procedure is as follows: first, a greater volume of blood can be withdrawn than is replaced, so relieving the raised venous pressure in severely anaemic cases. Secondly, nearly all of the infant's rhesus positive cells which are being lysed by the antibody are removed; they will not be replaced from the erythropoietic centres for some time unless the anaemia persists. Thirdly, the antibody which is damaging the cells is also almost completely removed and cannot be supplemented. Fourthly, a large part of the bile pigments responsible for kernicterus are removed, though they may reaccumulate as a result of diffusion from the tissues or because of further haemolysis or from both these causes. Fifthly, the replacement with rhesus negative blood provides the infant with sufficient oxygen-carrying capacity until the antibody is excreted and a new population of the infant's own cells [illegible] Finally the administration of [illegible]

be fresh so that as few as possible of the cells break down early in the infant's circulation thereby worsening the difficulty of bile pigment excretion, and it should be "partly-packed" to give as many cells as possible without overloading the infant's circulation. The technique of exchange transfusion using the umbilical vein is described by Mollison (1956 *a*) and Walker and Neligan (1955).

Exchange transfusion has been extremely successful in reducing the mortality of haemolytic disease. Many infants, however, are so mildly affected at birth that no treatment is necessary, particularly when anti-A or anti-B is the cause. It is obviously necessary to decide which infants should be treated, in other words, which are going to be severely affected. This decision depends on the appreciation that the infant is threatened with two possible causes of death, anaemia and kernicterus. The cord blood haemoglobin will determine the risk of the former, and it is general practice

. . . haemoglobin of less than 15 G. . . . no certain index of the . . . . . it may reflect the rate . . . it is unlikely to provide any information . . . liver function So it is necessary to consider the indications for transfusion in infants not unduly threatened with anaemia. There are . . . open One is to transfuse any infant which, . . . expected to develop severe . . . birth weight (below . . . ve been severely affected. Another . . . ht-hourly serum bilirubin estimations with a view to . . . in- . . . than 20 mgm if action is called for . . . course, recommended by . . . for haem pig- . . . transfuse . . . in significant . . . absorption maxima . . . ve to be repeated . . . ot estimate how soon liver efficiency . . . um bilirubin level is maintained below 20 mgm, the infant is likely to escape without harm. Boggs (1956) has suggested that it may be necessary to perform as many as four exchange transfusions in the first twenty-four hours if the level of haem pigment is to be controlled It is comforting to realize that with modern techniques exchange transfusion carries no significant hazards and that too frequent an application of this procedure is to be preferred to *laissez-faire*

It is evident that exchange transfusion can correct the anaemia without overloading the circulation It remains to consider whether the donor's blood suffices for the infant . . . cells are able to provide adequate oxygen car- . . . ml per pound body weight . . .

evidently be very . . . take into account the haematocrit . . .

[illegible] Walker and Neligan (1955), however, prefer [illegible]

[illegible] volume of fluid) the infant's anaemia will be corrected, its bone-marrow rendered quiescent and the rate of elimination of donor's cells such that the infant's own cells will not be called forth until most of the antibody has been excreted. Very rarely, or if the original exchange transfusion were inadequate, some anaemia may develop when the donor's cells are eliminated; there is some evidence that lysis of [illegible] after transfusion of com- [illegible]

Normally this second transfusion should not be called for, but it is desirable to follow the infant's haemoglobin level for several weeks after birth in order to provide this treatment if it is required.

Shocked and very anaemic babies will require appro- [illegible] of oxygen, the [illegible] transfusion have not been completed. The venous pressure during exchange transfusion can always be measured by attaching a manometer to the umbilical catheter.

The administration of calcium gluconate may not be necessary if partly packed blood is used, though some authorities advise it The level of serum potassium may need to be watched, however, particularly in infants threatened with heart failure (Campbell, 1955). Vitamin K need not be given if transfusion is undertaken and should in any case be administered only in small doses when it is required, particular care being necessary in premature in- [illegible] bone marrow stimulant [illegible] olytic [illegible] s and [illegible] le to [illegible] n the transfused cells [illegible] urity without blood group incompatability. There are theoretical

objections to breast feeding in haemolytic disease, but in practice no harm seems to accrue.

The principles of treatment in other species differ from those in man. In the mule breeding industry the aim is to

vidual foal may be of great value and the pedigree may need to be preserved. Hence, two courses are open to the owner of an immunized mare. One is to search for a stallion that

neo-natal period, exchange transfusion offers the best hope of recovery. The principles are the same as those that apply to the human infant except that the risk of kernicterus can be ignored. It is necessary only to adapt the calculations so

might cost more than the value of the relatively few piglets that succumb. A minor modification which might reduce the incidence in Britain would be to prepare the vaccine from the blood of Essex and Wessex breeds only; or from herds of suitable blood groups when these come to be known Fostering susceptible piglets on to other sows is not simple or successful, and changing the boar is unlikely to ameliorate the situation. Once a sow starts to produce litters that do not survive, it is best sold.

A NOTE ON NOMENCLATURE

The term *haemolytic disease of the newborn* has come to be preferred to *erythroblastosis foetalis*. The latter term, though it has enjoyed longer use, lays perhaps a little too much emphasis on the regenerative phenomena that follow the condition in man, though not always in other species, rather than on the blood destruction that initiates it; *haemolytic disease* puts one in mind of the icteric as well as the anaemic sequelae. Moreover, the emphasis on the foetus is possibly unfortunate for, though the condition may start *in utero* in man and in the rabbit, the clinical manifestations are seen, principally in those species and exclusively in others, in the neo-natal period.

The term *incomplete antibody* may well prove the least [illegible]

[illegible]

vantage here, in being expressed in English without the [illegible]

[illegible]

The word *conglutinin* and its derivatives has been eschewed so far as it might relate to blood grouping techniques, for fear of confusion with the original use of the term, coined by Bordet in the early years of the century, referring to the action of a heat-stable component of ox serum in clumping cells that have combined with both anti-

body and complement. Bordet's conglutination technique has so far found no useful application in blood group work other than among cattle.

The term *antiglobulin test* or *reaction* seems a suitably descriptive name for this technique, though *Coombs' test* for the direct diagnostic test on the cells of the newborn infant is quite convenient. Dr Coombs himself uses *antiglobulin sensitization test*, which is even more descriptive but rather a mouthful for daily use.

The word *immunized* has been used to indicate the evolution of antibodies by an animal *Iso-immunization* refers to the production of antibodies against an antigen *found in* other members of the same species The word *sensitized* is *here used solely for cells, to indicate that they* have antibody adsorbed on to their surface. The word *coated*, though more evocative, has not been used because of the implication that the cell surface is covered or invested by antibody molecules, which may well not be true.

In view of the criticisms of Kabat (1956) on the use of the terms *natural* and *immune* antibodies, it might be as *well to indicate a commonly accepted interpretation* of these terms *Natural antibodies* are those normally discoverable in the serum of any species which are present throughout most of its life and for which no known antigenic stimulus can be specifically identified. The term is not intended to imply that these antibodies have necessarily arisen spontaneously or genetically, or in the proved absence *of any antigenic stimulus, homogenetic or heterogenetic,* whatsoever. *Immune antibodies* are those that have arisen from specific, definable and often observed antigenic stimuli Sometimes these immune antibodies exhibit certain serological differences from the natural ones as, indeed, McDuffie and Kabat have themselves shown Moreover, Winstanley *et al* (1957) have shown that, with human anti-A, *there is a difference of specificity These differences* may not be consistent throughout all iso-antibody systems studied, but, as they are of obvious importance in the biological studies of haemolytic disease, it is convenient to be able to refer to them by existing nomenclature until our improved *knowledge permits of a better.*

[illegible]

accustom themselves to some of these proposals. I regret that I have adhered to the old-fashioned, but I hope well-established, usage of to *suckle*, indicating an activity of the mother, and to *suck* and (perhaps less common) to *nurse*, denoting an activity of the young.

APPENDIX

1. Panaroli's case (1654): "Infantum Barbitonsoris filium ictericum natum vidimus cum magno stupore qui postea *placente Deo evasit et nunc optima fruitur valetudine.*"

2. *Horst's case (1673) in translation:* "Recently a most noble and beautiful woman of warm and humid disposition, thirty-two years old, who was six-months pregnant and at a time when malignant petechial fevers were rife, experienced severe and frequent labour pains without any immediate cause being evident, and, with a great flux of water and blood from the uterus, brought forth a well-formed but stillborn foetus entirely jaundiced, together with a false conception and a very livid after-birth . Since this woman had already twice borne sons which died of jaundice within ten days of birth, I dissected this dead foetus, on the instruction of those that stood by, and found not only that the stomach and intestines were full of bile, but also that the liver was dry, the spleen hard, the lungs corrupted and the heart flaccid. sufficiently obvious causes of death "

3. Schultze's case was published in "Miscellanea curiosa medico-physica Academiae naturae curiosorum sive Ephemeridum medico - physicarum Germanicarum " Annus VI and VII, covering the transactions for 1675 and 1676 does not appear to have been published until 1688. The page reference is sometimes given, erroneously, as 355 The cases of Fehre and Anhorn are given in "Miscellanea curiosa sive Ephemeridum medico-physicarum Germanicarum Academiae Caesareo-Leopoldinae naturae curiosorum " This is a continua-

tion of the same journal under a different title. The case described by Michael (1688) is Horst's case. [illegible] tury).

4 "This distemper for the most part seizes infants presently after birth; very often they are born infected with it and therefore it may be supposed they contain the cause of it within themselves without being infected by the nurse; and it is probable that it owes its origin to a glutinous and sizy Humour, insomuch that it may often happen without any obstruction of the biliary duct, although this obstruction is the cause of the jaundice in adult or full-aged persons. . . . I do not remember many practical authors . who have taken notice of the jaundice in infants, nevertheless, many die of it for want of proper and seasonable helps; and most nurses are so stupidly ignorant that they imagine because the poor child grows yellow—consequently it must die, and therefore they will not look out for help."

5 Among dissertations that have been consulted are:

Bidault, F (1804) "Essai sur l'ictère des nouveau-nés" Paris.

Vaillant, L. (1806) "Sur l'ictère des nouveau-nés." Paris

Bourgeois, L J. (1817) "De ictero neonatorum." Lugduni Batavorum.

Denis, P S (1824) "De L'ictère et de l'endurcissement du tissu céllulaire des enfans nouveau-nés" Paris

Leger, T (1825) "Considération sur l'endurcissement du tissu céllulaire chez les nouveau-nés"

Bouton, J P (1838) "De l'ictère des nouveau-nés" Paris

Hervieux, J F E. (1847)· "De l'ictère des nouveau-nés." Paris

Porchat, A. (1855) "De l'ictère chez les nouveau-nés." Paris.

Moreau, J. A. E. (1858). "De l'ictère chez les nouveau-nés." Paris.

Gaste, J. B. L. (1859) "De l'ictère des nouveau-nés" Paris.

Michel, L. E. (1867). "De l'ictère des nouveau-nés." Paris

The "endurcissement" referred to does not seem to be hydrops foetalis.

6. Pearson's case as described in Underwood is as follows:

"Mrs. J. had been the mother of eleven children: in nine of which the jaundice appeared a few days after they were born, and they all died within the period of a month after their birth The tenth child lived six years, was then afflicted with the jaundice, and died In May 1796, Mrs. J. was delivered of her eleventh child; on the third day after its birth, the skin became yellow, and the child was at the same time remarkably torpid and sleepy, and seemed to be slightly convulsed. On the following days the colour of the skin often varied, being sometimes of a deeper yellow, and at other times nearly regaining its natural colour, the child continued, however, in the same languid and almost insensible state, but received nourishment, and sucked the breast of its mother, till within a few hours of its death, which took place on the ninth day I opened the body of this child the day after it died, and shall now proceed

whole concave surface of the right lobe had a livid

margin of both the lobes; there the thin edge exhibited a highly injected appearance; the redness was, however, less vivid and remarkable on the left lobe than on the right. There was also a slight adhesion of the lower part of the right lobe to the peritonaeum The gall bladder was nearly filled with bile of a deep yellow colour; and its ducts were permeable. The stomach was in a natural state, and the intestines were without any marks of disease. In the thorax, the lungs were of a healthy appearance. [illegible]

water"

7 [illegible]

it is the same book The fourth edition, which is the [illegible]

8 J Cheyne wrote three "Essays on the Diseases of Children". The first essay on Croup was published in 1801 The second on "The bowel complaints more immediately connected with the biliary secretion" was published in 1802, and is the one referred to in the text. The third essay was issued in 1815.

9 Congenital obliteration of the bile duct was reviewed [illegible]

are collected in the tables published severally by Smith (1855), Jenkins (1858) and Grandidier (1871). It is perhaps not quite fair to say that no one referred to Pearson or Cheyne until 1883 for a comment can be found buried in Frank's enormous work of 1843; but it is mixed up with other irrelevancies, and although the "hereditary" nature is mentioned, the point is confused with cases that are certainly not neo-natal. The book contains a fine bibliography however.

10. C. West, who was associated with the founding of the Hospital for Sick Children in Great Ormonde Street, first published his "Lectures on the Diseases of Infancy and Childhood" in 1848; but neither this nor the two following editions contains the passage quoted in the text, which is from the fourth edition of 1859. A further case, probably of haemolytic disease, is added in the fifth edition of 1865.

11 Louyse Bourgeois, called Boursier, was born in 1563 and died in 1636 The first three editions of her book were published in Paris in 1609, 1617 and 1626 In this last year the second edition was issued at Rouen. The fourth and fifth editions were published in Paris in 1642 and 1652

12 Louyse Bourgeois's case in translation·

"Of a lady whom I delivered of two children after seven months· the daughter was hydropic, but the son was not."

"A lady desired my services and sent for me. I went to see her one morning and found her dressing I have never seen such a belly as she had She ordered a servant of hers to prepare an enema for her, according to custom As she had a pain in her loins I gave her the enema and found that the pain in her loins did not lessen. I made her warm and soon saw that she was in labour. She told me that at the orders of M Martin, a very learned doctor, she had been bled six times on account of her pregnancy, had

taken three or four medicines and quite 100 enemas, as otherwise she would have become suffocated She was happily delivered, without much trouble, of a girl, who came head first. As she was coming out I felt a hardness such as reminded me of a child which M. du Laurens, first Physician to the King, said he had seen at Sens in Burgundy, at the premises of a surgeon, and which a woman bore at eighteen years of age, and it was as hard as a stone. I thought I was holding a similar child I saw a girl, alive and hydropic, from the head to the thighs and up to the lips, so hard that the child could not have been harder. It seemed as if one was touching wood. She had a fat belly, stretched like a ball, extremely black, and because of the tension there was not the smallest vein in it that was unbroken The child lived about a quarter of an hour Although she was very big I reflected that she was not big enough to have occupied the whole of that belly Noting her weakness, I tied and cut the umbilical cord and sent her to be held in front of the fire I then sent for the curate of St Leu St Giles for her to be baptized Having meanwhile felt the mother, I found the waters of another child on the point of breaking When they had broken, a little boy came out feet first He was strong and thick set, and lived some time When this little boy was born a great quantity of water came out of the mother, such as one had never seen I should say there was a bucketful It was so yellow that the linen soaked in it was stained by it I wanted M Martin to come and see the mother and the children, together with the evacuation, particularly because he had attended her during her pregnancy, so that he might see the cause of her ill He thought it a great gift of God that she had been delivered, and considered that the belly of the hydropic daughter was a real gangrene That was not the only occasion when I have recognized that Nature has a wonderful providential way of knowing how to rid herself of what is harmful to her, provided that the ill is con-

son or Cheyne until 1883 for a comment can be found buried in Frank's enormous work of 1843, but it is *mixed up with other irrelevancies, and although the*

10. C. West, who was associated with the founding of the Hospital for Sick Children in Great Ormonde Street, first published his "Lectures on the Diseases of Infancy and Childhood" in 1848; but neither this nor

added in the fifth edition of 1865.

11. Louyse Bourgeois, called Boursier, was born in 1563

12. Louyse Bourgeois's case in translation:

> *"Of a lady whom I delivered of two children after seven months the daughter was hydropic, but the son was not."*

"A lady desired my services and sent for me I went to see her one morning and found her dressing I have never seen such a belly as she had She ordered a servant of hers to prepare an enema for her, according to custom As she had a pain in her loins I gave her the enema and found that the pain in her loins did not lessen I made her warm and soon saw that she was in labour She told me that at the orders of M Martin, a very learned doctor, she had been bled six times on account of her pregnancy, had

girl, who came head first. As she was coming out I

lips, so hard that the child could not have been

held in front of the fire. I then sent for the curate of St Leu St Giles for her to be baptized Having meanwhile felt the mother, I found the waters of another child on the point of breaking When they had broken, a little boy came out feet first He was strong and thick set, and lived some time When this little boy was born a great quantity of water came out of the mother, such as one had never seen. I should say there was a bucketful It was so yellow that the linen soaked in it was stained by it. I wanted

are collected in ...
(1855 ... it is perhaps ... that no one referred to Pearson or ...yne until 1883 for a comment can be found buried in Frank's enormous work of 1843; but it is mixed up with other irrelevancies, and although the "hereditary" nature is mentioned, the point is confused with cases that are certainly not neo-natal. The book contains a fine bibliography however.

10 C. West, who was associated with the founding of the Hospital for Sick Children in Great Ormonde Street, first published his "Lectures on the Diseases of Infancy and Childhood" in 1848; but neither this nor the two following editions contains the passage quoted in the text, which is from the fourth edition of 1859 A further case, probably of haemolytic disease, is added in the fifth edition of 1865.

11. Louyse Bourgeois, called Boursier, was born in 1563 and died in 1636. The first three editions of her book were published in Paris in 1609, 1617 and 1626 In this last year the second edition was issued at Rouen. The fourth and fifth editions were published in Paris in 1642 and 1652

12 Louyse Bourgeois's case in translation -

> *"Of a lady whom I delivered of two children after seven months the daughter was hydropic, but the son was not"*

"A lady desired my services and sent for me. I went to see her one morning and found her dressing I have never seen such a belly as she had She ordered a servant of hers to prepare an enema for her, according to custom. As she had a pain in her loins I gave her the enema and found that the pain in her loins did not lessen I made her warm and soon saw that she was in labour She told me that at the orders of M Martin, a very learned doctor, she had been bled six times on account of her pregnancy, had

tion marks not present in the original; and the last word should read "desinebat".

14. [illegible]

p. 205.

15 The following references of Ballantyne were not consulted:

Osiander, Carus, Lamouroux, Meissner, Olivier, Graetzer, Hohl, Weber, Ferris, Goldmann, Clay, Ritter, Herrgott, Franck, Strauch, Braums, Ahlfeld, Honck, Pinzani, Lohlein, Taruffi, Gueniot, Fuhr, Dareste, Raineri, Betschler and Mattersdorf.

From Ballantyne's own comments on these reports few of them are likely to have been cases of haemolytic disease Among those that are considered as possible cases of haemolytic disease are·

Plater, Dorstenius, Schurig, Billard, Senlen, Simpson, West, *Montgomery*, *Burton*, *Smith*, *Beck*, *Bassett* and Ballantyne's own cases

The rest are either extremely doubtful or easily rejected

16 The critical sentence in Halban's paper reads

"Es ensteht nun die Frage woher diese Idioisoagglutinine stammen und es lag der Gesdanke nahe—und dies war auch der Ausgangspunkt meiner Arbeit—, ob nicht das Auftreten der Idioisoagglutinine im mutterlichem und fotalen Blute als der Ausdruck einer wechselseitigen Immunisirung von Mutter und Frucht aufzufassen ist"

17 [illegible]

18 Kirronosis was a yellow staining of the body, including the brain and spinal cord, in five-month foetuses.

fined to some place from which it can make an exit. *I shall never again be astonished at dropsies of the* womb, for I have seen a number from which the mothers of all, and the majority of the children, have escaped. I showed *the placenta of these two children* to M. Martin. There was only one for the two of them, with the usual separation between them. The *girl's placenta was full of a yellow mucus, and the* veins through which the nourishment was carried to her umbilical vein were full of yellow blood, like *that which one normally draws from a pleuritic. The* boy's placenta was fine and clean. The veins by which his nourishment was carried were big and full *of fine crimson blood. I saw him two or three days* after he was born. He had turned yellow, as most children normally do, and possessed all his natural *functions But he had so much phlegm that, in spite* of all efforts, it was impossible to save him. All the ill of the mother and her children came (by her own *confession) from drinking. She said that* at one meal she had drunk quite three chopins of water, because she had such a great thirst. I have often heard physicians say that such excesses came from a heated liver, as indeed the jaundices show which were in the blood and in the waters "

13 Felix Plater's case in translation:

"*I have encountered a case of an immature* male foetus, produced by an abortion at the fourth or fifth month, which was already *hydropic* in its mother's uterus—I examined its contents and found the skin of the whole body and the hairy part of the scalp to be *distended with water, and from the base of the* bladder was a wide channel the size of the urachus, ending below the kidneys in the vena cava—this was *filled throughout with serosanguineous material*"

The first edition of Plater's book was published in 1614, and the second in 1641, hence Ballantyne's error *in dating which has been copied subsequently* The transcription given by Pickles (1949) carries punctua-

[illegible]

be consistent with the plasma pigments observed in kernicterus by Tuttle (1955) and Abelson and Boggs (1956), with absorption maxima in the regions of 620 mμ and 405-415 mμ; the latter authors thought that the nearer the maxima approached 405 mμ the [illegible]

[illegible] and Boggs, 1956)

It may be, however, that no trouble arises while the porphyrin ring remains closed, but that once it is [illegible]

The chemistry of the dipyrrolic substances, and the dipyrrylmethenes particularly, is even more obscure. Characteristically the group shows two absorption maxima in the regions of 450 and 250 mμ Further knowledge of the properties of mesobilifuscin in particular would be welcome

Apart from the natural difficulties in recognizing and distinguishing these three groups of compounds, it seems likely that mixtures of considerable complexity are present in neo-natal jaundice and the mutual effect on their physical and chemical properties cannot yet be worked out

23 The incidence of kernicterus has been variously estimated A selection of figures may be given: 4% of all

The *pigment* was unaffected by exposure to water, alcohol or light. The condition is admirably illustrated in the original paper. I have met with no more recent description.

19. [illegible]

first French edition.

20 Many papers have been *published* about icterus neonatorum (icterus praecox) *and related topics*, including ABO heterospecific pregnancy generally, in the last twenty years. Some of these deal with the relationship of *jaundice* as a symptom to *heterospecific pregnancy* or to the serological consequences of heterospecific pregnancy, in others *bilirubinaemia* rather than jaundice is the criterion selected for the investigation of *similar relationships*. Or it may be that *the serological* and haematological *consequences* of heterospecific [illegible]

Obrinsky, Allen and Anderson *(1954)*, which has a full bibliography; *Zuelzer* and *Kaplan (1954), a most* comprehensive *study*, *Hsia, Allen, Diamond and Gellis (1953)*, *Johnstone* (1953) and *Billing, Cole and Lathe (1954)*

21 Some papers *on this topic are* Karsner and Aub (1913), liver only; *Mella (1924)*; Baló and Korpássy (1932), Fuchs (1914), Crandell and Weil (1933), Kirschbaum (1922), Nicolaiev (1937), and Guillain, *Fiessinger, Mollaret and Delay (1938)* A *full* bibliography is *given* by Polani *(1954 b)*

22 *The problems will no doubt become clarified by advances in the knowledge of bile pigment chemistry* The normal course of haemoglobin breakdown, for example, is far from clear If the first stage is the loss of

of trypsin-modified cells or by haemolysis in an acidified medium or using trypsin-modified or paroxysmal nocturnal haemoglobinuria cells Those demonstrated

probably of normal occurrence and anti-H specificity.

5 Villa Roya's account has not been seen. The earliest citation of the account is that of Bouley and Reynal (1874), but they do not give the reference to Villa Roya's century-old work; later authors have copied Bouley and Reynal The Proceedings of the Agricul-

Some of the accounts of this condition in mules, which

26

admissions to a prematurity unit; 8·8% of infant necropsies, of which 73% were due to iso-immunization; 18% of all cases of haemolytic disease; 25 of 239 infant necropsies, 144 of them being of premature infants, 10 of the cases being due to haemolytic disease.

There are a number of detailed clinical and pathological accounts of kernicterus, among which are those of Beneke (1907); de Lange (1935); Gilmour (1944); Bertrand (1946), Pentschew (1948); Dereymaeker (1949); Vaughan, Allen and Diamond (1950); Evans and Polani (1950); Claireaux (1950), Gerrard (1952), and Bertrand, Bessis and Segana-Obiol (1952).

24. The circumstances referred to occur when the cells are sensitized by auto-antibodies, i.e. antibodies present in *a patient's serum that are specific for his own cells.* The situation regarding auto-antibodies is very confused and yet it touches the arguments in Chapter IV at many points. The position is admirably summarized by Dacie (1954), who has himself contributed very materially to an understanding of the matter. Some *auto-antibodies are referred to as "warm" antibodies* because body heat favours sensitization. They are thought to be gamma globulins in the majority of cases, and sometimes they show a distinct specificity for one of the blood group antigens (usually connected with the Rh system), but they may appear to be non-specific or be accompanied by a non-specific component. They may be demonstrated by an irreversible agglutination of trypsin-treated cells, particularly if serum is used as a diluent Their activity, however, may be expressed by haemolysis if either the testing medium is slightly acidified or if trypsin-modified cells are used; cells from patients with paroxysmal nocturnal haemoglobinuria are also effective The presence of these warm auto-antibodies is usually associated with some pathological condition such as acquired haemolytic anaemia

By contrast, other auto-antibodies are called "cold" antibodies because sensitization is maximal at refrigerator temperature. They are usually harmless and

Hedal's G and a respectively; these three antigens are [illegible]

and Cohen (1956) has identified others which he calls B, C, E and H, one of which may be Heard's X. It is to be hoped that the nomenclature of these can be further rationalized when genetic data are available The variations in agglutinability reported by Kellner and Hedal and Heard has been ascribed by Heard (1955 *b*) to a variation in the *number of antigen sites* on the cell [illegible]

28. A report of haemolytic disease has appeared in Italy (Romagnoli *et al*, 1956) and a second report from America (Newberne, Robinson and Rising-Moore, 1956) In this last the influence of swine fever vaccination is admitted. A note added by the Editor refers to a case reported in 1932, five months after the sow's vaccination
29. This matter has been discussed again recently. In an [illegible]

natally, and a step towards its solution has been made by Murray (1957), who shows that the prognosis may depend in part on the father's Rh genotype (i e. the composition of Rh antigens)

This observation of Murray has far greater importance than mere prognosis It suggests that D is more

fuller account of their experiences of this disease comprising sixty litters

Their work on blood groups concerned chequer-board tests involving the serum and cells of 1,120 pigs, together with the appropriate absorptions. By this means they were able to identify four antigens which they called A, B, C and D (A being known previously and C proving the least common of the four), and twelve of the sixteen possible combinations of these antigens (O was found, but ABCD, ABC, ACD and BCD were not). This work was based entirely upon the use of naturally occurring antibodies, no immune sera being used, the frequency of these natural antibodies is low, only about a third of the animals possessing them.

Szent-Iványi and Szabó also observed, as Saison, Goodwin and Coombs (1956) had done, that some samples of group A red cells absorb anti-A from an antiserum even though they will not be agglutinated by that antiserum; and this property did not appear to be confined to the young piglet It is evident that further pig blood group antigens would be revealed by the use of immune antisera, and the Hungarian authors are in doubt whether the "Su" antigen and antibody, which they described in their first report as responsible for haemolytic disease, is identical with any of the four antigens and antibodies which they later demonstrated

Rabbit blood groups have been the subject of much study recently Kellner and Hedal studied haemolytic disease with a pair of allelomorphic antigens which they termed G and g, and which they believed were different from Castle and Keeler's H_1 and H_2, partly because rabbits are known which possess neither H_1 nor H_2, but Kellner and Hedal could find none possessing neither G or g However, Anderson (1955) and Joysey (1955) found a third allelomorph and suggested that both systems were the same The latter author proposed a nomenclature for the three allelomorphs G^a, G^b G^c, the first two representing Kellner and

REFERENCES

Abelson, N. M., and Boggs, T. R. (1956). "Plasma pigments in erythroblastosis fetalis. 1. Spectrophotometric absorption patterns 2. The level of heme pigment, an early guide to management of erythroblastosis fetalis", *Pediatrics*, Springfield, 17, 452, 461.

Adamson, C. A., Lofgren, S., and Malmonäs, C. (1951). "Antibodies in mothers and newborn infants", *Scand. J. clin. Lab. Invest.*, 3, 52

Aidin, R., Corner, B., and Tovey, G. (1950). "Kernicterus and prematurity", *Lancet*, 1, 1153

Allen,

III.

mat

Springfield, 6, 441.

Allen, W. M. (1926) "Interagglutination of maternal and fetal blood in the late toxaemias of pregnancy", *Johns Hopk. Hosp Bull.*, 38, 217.

Allison, A. C. (1955) "Danger of Vitamin K to Newborn",

Med., 41, 615.

Anderson, C F. (1953) "Haemolytic disease in a first-born infant", *Brit med J*, 1, 1032.

Anderson, J. R. (1955) "Blood groups in rabbits", *Brit. J. Haemat*, 1, 378.

Anderson, J. R. (1956) "The experimental production of erythroblastosis foetalis in rabbits", *Brit J Haemat*, 2, 44

Anderson, W C. (1850) "On haemorrhage from the umbilicus after the separation of the funis", *Boston med. surg. J.*, 41, 440

antigenic when associated with E than when with C. Hence the incidence of immunization is higher against cDE than against CDe, and hence, too, the "antigenicity" of the blood group is a material factor in this disease. Murray's explanation is that since E is thought to be a weaker antigen than C, and since the amount of blood group material available for C, D and E is limited, more would be available for D if its partner were E than if it were C. To illustrate it numerically, let us suppose that the total antigenic material available for C, D and E would provide for 20x antigen sites; C would normally be expressed by, say, 6x—so that 14x would be available for D in a CDe/cde person. But E might normally be expressed by only 3x sites, leaving 17x for D in a cDE/cde person. And it would be assumed that the difference between 17x sites and 14x sites would be reflected by a materially greater antigenicity.

There might, of course, be other explanations, but this one could possibly account also for the greater severity in cases in which D is associated with E rather than with C. More antibody would b[illegible]
more antigenic f[illegible]
bound by t[illegible]
form of an [illegible] sites or some similar form. If the cells bind more antibody they might be destroyed more quickly or more certainly.

Another comparable recent observation is that of Winstanley *et al* (1957), showing that the A antigen that provokes the formation of "immune anti-A" is of different specificity from that which is recognized by "natural" anti-A. Perhaps the latter is always neutralized, and immunization occurs only when the former is present. This needs to be worked out. Both these observations provide important clues to the genesis of haemolytic disease.

Behnsen, G. (1927) "Ueber die Farbstoffspeicherung im Zentralnervensystem der weissen Maus in verschiedenen Altersuständen", *Zeitschr. f. Zellforsch. u. mikr. Anat.*, 4, 515.

Beijers, J. A., van Loghem, J. J., and van der Hart, M. (1950).

no-
v.
Diergenees, 76, 711.

Belfanti, S. and Carbone, T. (1898). "Produzione di sostanze

Bertrand, I (1946). "Lésions du système nerveux central dans deux cas d'ictère nucleaire du noveau-né", *Rev. d'Hématol*, 1, 399.

Bertrand, I., Bessis, M, and Segana-Obiol (1952) "L'ictère nucleaire" Masson, Paris.

Bessis, M. (1947). "La maladie hemolytique du noveau-né" Masson, Paris

Bessis, M., and Caroli, J (1947). "Anticorps anti-mulets incomplets et bloquants chez la Jument mère de muleton icterique", *C. R Soc. Biol*, 141, 387

Bessis, M, and M
du colostrum c
muleton nouve

Bevis, D C A (1
disease of the r

Bierling, C. T. (1694) "Thesaurus theoretico-practicus", p. 1076, para 89. Jena.

Anderson, J. F., and Rosenau, M. J. (1908). "Further studies upon anaphylaxis", *J. med. Res*, 19, 37.
Andrews, H. R. (1901). "Two cases of foetal ascites and oedema", *Trans. obstet. Soc. Lond*, 43, 166.
Anhorn, S. S. (1693). "De Infante recens nato, ab extremorum in aquam frigidam immersione Icterico, brevique mortuo", obs. 86 on p. 134 in "miscellanea curiosa sive Ephemeridum medico-physicarum Germanicarum Academiae Caesareo-Leopoldinae naturae curiosorum", Decuriae III, Annus I. Frankfurt and Leipzig.
Arkwright, J. A. (1902) "A family series of fatal and dangerous cases of icterus neonatorum: fourteen cases in one family with four survivors", *Edinb. med J., N S*, 12, 156.
Ascoli, A. (1902) "Zur experimentallen Pathogenese der Eklampsie", *Zentralb. f. Gynäk*, 26, 1321.
Ashby, H. (1884) "Some fatal cases of icterus neonatorum", *Med. Chron.*, 1, 25. See also Ashby, H., and Wright, G. A. (1889), "The Diseases of Children", pp 24 and 25
Auden, G. A. (1905). "A series of fatal cases of jaundice in the newborn", *St. Bart's Hosp Rep.*, 41, 139.
Ayrault, E (1867). "De l'industrie mulassière en Poitou." *Niort, p. 175.*
Baar, H. S (1945). "Hyperchromic anaemia", *Austrian Medical Bulletin*, Oct -Nov , p 1.
Baar, H. S (1946). "The post-mortem examination of the newborn infant", *Brit Med Bull.*, 4, 178.
Bakay, L (1956). "The Blood-brain Barrier." Thomas, Springfield Pp 80 *et seq.*
Ballantyne, J W (1892) "The Diseases and Deformities of the Foetus " Edinburgh
Ballantyne, J W. (1902) "Manual of Antenatal Pathology and Hygiene, the Foetus " Green & Sons, Edinburgh, p. 363
Baló, J , and Korpássy, B. (1932) "The encephalitis of dogs with Eck fistula fed on meat", *Arch. Path.*, 13, 80.
Banssillon, E (1929) "Signification clinique et thérapeutique des cas d'agglutination réciproque des globules rouges de la mère et de l'enfant nouveau-né", *J Méd. Lyon*, 10, 637
Barcroft, J. (1946) "Researches on Pre-natal Life " Oxford
Barnet (1931) "Un cas de jaunisse du muleton", *Rev vét*, 83, 29.
Barr, M , Glenny, A T , and Randall, K J (1949) "Concentration of diphtheria antitoxin in cord blood and rate of loss in babies", *Lancet*, ii, 324.
Baumes, J. B. F (1788). "Traité de l'ictère des enfans de naissance " Paris.

Behnsen, G. (1927). "Ueber die Farbstoffspeicherung Zentralnervensystem der weissen Maus in verschied Altersuständen", *Zeitschr. f. Zellforsch. u. mikr. Anat.* 515

Beijers, J. A., van Loghem, J. J., and van der Hart, M (19 "Haemolytische Anaemie bij het pasgeboren veulen", *Tijd v. Diergenees*, 75, 955.

Beijers, J. A., van Loghem, J J, and Borstel, H. (1951) "Hae lytische Anaemie bij het pasgeboren veulen", *Tijdsch Diergenees*, 76, 711.

Belfanti, S and Carbone, T. (1898). "Produzione di sost

E

E tionen und infektionen. *Verhandl. Deutsch. Path. Gesellsch.* 403.

Bernadin. See Sanson (1863)

Berry, G P, and Slavin, H. B. (1943) "Studies on he

1, 399

Bertrand, I, Bessis, M, and Segana-Obiol (1952). "L'i nucleaire" Masson, Paris.

Bessis, M (1947) "La maladie hémolytique du noveau- Masson, Paris

Bessis, M., and Caroli, J (1947) "Anticorps anti-m incomplets et bloquants chez la Jument mère de mul ictérique", *C R. Soc Biol*, 141, 387

para. 65, Jena

Billard, C. (1828). "Traité des Maladies des Enfans nouveau-nés et à la mamelle." Paris.

Billing, B. H., Cole, P. G., and Lathe, G. H. (1954). "Increased plasma bilirubin in newborn infants in relation to birth weight", *Brit. med. J.*, ii, 1263.

Bingold, K. (1935). "Ueber die Bedeutung von Katalase und Hydroperoxyd für den Blutstoffwechsel. Darstellung eines biologischen Blutfarbstoffabbaues auf Grund von Modellversuchen", *Deutsch. Arch. f. klin. Med.*, **177**, 230.

Binz, C. (1866). "Zur Kenntniss des tödlichen Icterus der Neugebornen aus Obliteration der Gallengänge", *Arch f. path Anat. u Phys. u. f. klin. Med.* (*Virchow's Archives*), **35**, 360.

Blomfield, J. E. (1901). "Congenital hepatic cirrhosis", *Brit. Med. Jour.*, i, 1142.

Boggs, T. R. (1956). "Exchange transfusion as a therapeutic measure with special reference to its use in erythroblastosis fetalis", *Progress in Haematology*, vol. 1, p. 47.

Bonnin, J. A., and Schwartz, L. (1954). "The combined study of agglutination, haemolysis and erythrophagocytosis", *Blood*, **9**, 773.

Booth, P. B., Dunsford, I., Grant, J., and Murray, S. (1953). Correspondence. *Brit. Med. Jour.*, ii, 41.

Bouchet, N. du (1926). "Interagglutination positive malgré l'identité de groups entre le sang de la mère et du nouveau-ne", *C R Soc. Biol.*, **94**, 16.

Bouley, H. (1863). Cited by Gauchot, 1936.

Bouley, J., and Reynal (1874). "Nouveau Dictionaire pract. de méd et dé chir et d'hyg vét." Tom. IX, p. 10, article on "Haematurie".

Bound, J. P., and Telfer, T. P. (1956). "Effect of vitamin-K dosage on plasma-bilirubin levels in premature infants", *Lancet*, i, 720.

Bourgeois, L. (1609). "Observations diverses sur la sterilité, perte de fruict, foecondité, accouchements, et maladies des femmes, et enfants nouveaux-naiz" Paris.

Boursnell, J. C., Coombs, R. R. A., and Rizk, V. (1953). "Studies with marked antisera. Quantitative studies with antisera marked with iodine ^{131}isotope and their corresponding red-cell antigens", *Biochem. J.*, **55**, 745.

Boursnell, J. C., Heard, D. H., and Rizk, V. (1955). "Studies with marked antisera. 2. Variation in the specific adsorption by red cells from different rabbits of one ^{131}I-marked rabbit isoantiserum", *J. Hyg. Camb.*, **53**, 420.

Bracken, H. (1737). "The midwives companion", Book 3, Ch. 6, p. 222, London.

Brading, I., and Walsh, R. J. (1954). "The role of tissue antigens in haemolytic diseases of the newborn", *Aust. J. exp. Biol. med. Sci*, 32, 213.

Brambell, F. W. R., Brierley, J., Halliday, R, and Hemmings, W. A. (1954). "Transference of passive immunity from mothe to young", *Lancet*, i, 964.

Brambell, F. W. R., and Halliday, R. (1956). "The route by [illegible]

Biol, 8, 476.

Brambell, F. W. R., Hemmings, W. A., and Henderson, M. [illegible]

anticorps antimulet chez son muleton", *Acad. Sci*, 228, 1614.

Brion, A. (1949 *b*) "Sur l'ictère hémolytique de Poulain nouveau-né", *Rev. méd. vét*, 100, 229.

Brion, A (1950) "Réalisation experimentale de l'ictère du Muleton nouveau-né", *C R. Acad Sci.*, 230, 1547.

Brion, A. (1951) [illegible] de la [illegible] *Soc.* [illegible]

325.

Brion, A., Richard, G, and Laffolay, B. (1951) "Résultats d'une campagne de prophylaxie de l'ictère des muletons", *Bull acad. vét*, 24, 165

Bruner, D. W. (1950). "Diagnosis of haemolytic icterus in foals", *Cornell Vet*, 40, 11.

Bruner, D. W., Brown, R. E., Hull, F. E., and Kinkaid, A. S. (1949) "Blood factors and baby pig anaemia", *Jour. Amer. Vet. Med. Ass.*, **115**, 94.

Bruner, D. W., Doll, E. R., Hull, F. E., and Kinkaid, A. S (1950). "Further studies on baemolytic icterus in foals", *Amer. Jour. Vet. Res.*, **11**, 22.

Bruner, D. W., Edwards, P. R., and Doll, E. R. (1948) "Passive [illegible]

Br[illegible] relation [illegible] *et. Res*, [illegible]

Bruner, D. W., Hull, F. E., Edwards, P. R., and Doll, E. R. (1948) "Icteric foals", *Jour. Amer. Vet. Med. Ass.*, **112**, 440.

Brunschwig, A. E. (1927). "Notes on experiments in placental permeability", *Anat. Rec.*, **34**, 237.

Bryce, L. M , and Burnet, F. M. (1932). "Natural immunity to staphylococcal toxin", *J. Path. Bact.*, **35**, 183.

Bryce, L M., Jakobowicz, R , Graydon, J. J., and Campbell, K. (1951). "The incidence and effects of Rh incompatibility between mother and child", *Med. J. Aust* , **1**, 781.

Buchan, A. H., and M'Gibbon, J. (1906). "A case of congenital anaemia associated with jaundice", *Scot Med. and Surg. Jour* , **19**, 233

Buchan, A. H., and Comrie, J. D. (1909). "Four cases of congenital anaemia with jaundice and enlargement of the spleen", *J Path Bact.*, **13**, 398.

Buhrman, W L., and Sanford, H N. (1931) "Is familial jaundice of newborn infants erythroblastosis?", *Amer. J. Dis Child.*, **41**, 225

Burns, J. (1824) "The Principles of Midwifery including the Diseases of Women and Children," p. 796 6th edition. London.

Busfield, J (1906). "A series of cases of icterus neonatorum in a family", *Brit. Med J.*, **1**, 20

Butler, N. R , and Spector, W. G. (1952). "Kernicterus without prematurity or blood group incompatibility", *Brit Med. J.*, **1**, 1168.

Buxton, J C., and Brooksbank, N H. (1953 *a*). "Haemolytic disease of new-born pigs caused by iso-immunisation of pregnancy", *Nature*, (*Lond.*), **172**, 355.

Buxton, J C , and Brooksbank, N. H (1953 *b*). "Haemolytic disease of new-born pigs caused by iso-immunisation of pregnancy", *Vet Rec* , **65**, 287.

Buxton, J. C., Brooksbank, N. H., and Coombs, R. R. A. (1955). "Haemolytic disease of newborn piglets caused by maternal iso-immunisation", *Brit. vet. J* , **111**, 463.

Cadeac, P. (1896). "Pathologie interne (Sang)."
Campbell, A. D. (1844). "Contributions to infantile pathology; two cases of icterus gravis infantum from deficiency of the [illegible]
xii.
Capon, N. B. (1922) "General oedema of the foetus", *J. Obstet. Gynaec*, *Brit Emp*, 29, 239.
Capon, N. B. (1955) "The paediatrician's part in the maternity services", *Brit med. J.*, 1, 803.
[illegible]
114, 909.
[illegible]
rev. du Midi, 3, 330.
Castle, W. E., and Keeler, C. E. (1933). "Blood group inheritance in the rabbit", *Proc. Nat Acad. Sci.*, 19, 92
Chavasse, F. B. (1921) "The blood group in infants and their mothers" (report of a paper read to Liverpool Medical Institution), *Brit. Med J*, 1, 641
Cheselden, G (1729) Cited in *Brit med. J.*, 1953, 1, 558.
Cheyne, J. (1802). "Essay on the Diseases of Children." Essay II [illegible]

Chillaut, A. (1925). "L'ictère des poulains et des muletons nouveau-nés" Thèse. Toulouse.
Chown, B. (1954 *a*). "Anaemia from bleeding of the fetus into the mother's circulation", *Lancet*, **i**, 1213.
Chown, B. (1954 *b*). "Three unusual cases of anemia of the newborn". Discussion. *Amer. J. Dis Child.*, **88**, 508.
Chown, B (1955). "On a search for rhesus antibodies in very young foetuses", *Arch. Dis. Childh*, **30**, 224.
Christian, R. M., Ervin, D. M, Swisher, S M., O'Brien, W. A., and Young, L. E. (1949) "Hemolytic anemia in newborn dogs due to absorption of isoantibody from breast milk during the first day of life", *Science*, **110**, 443.
Christian, R. M., Ervin, D. M., and Young, L. E. (1951). "Observations on the in vitro behaviour of dog isoantibodies", *J. Immunol.*, **66**, 37.
Christian, R. M., Stewart, W. B., Yuile, C. L, Ervin, D. M., and Young, L. E. (1951) "Limitation of hemolysis in experimental transfusion reactions related to depletion of complement and isoantibody in the recipient; observations on dogs given successive transfusions of incompatible red cells tagged with radio-active iron", *Blood*, **6**, 142.
Claireaux, A (1950) "Haemolytic disease of the newborn Part II, Nuclear jaundice (Kernicterus)", *Arch. Dis. Child*, **25**, 61.
Claireaux, A E., Cole, P G., and Lathe, G H. (1953). "Icterus of the brain in the newborn", *Lancet*, **ii**, 1226.
Coffin, S F, and Pickles, M. M. (1953) "The ability of trypsin to restore specific agglutinating capacity of erythrocytes treated with periodate", *J. Immunol*, **71**, 177.
Cohen, C (1956) "Occurrence of three blood cell antigens in rabbit as the result of interaction of two genes", *Science*, **123**, 935.
Cole, P G, and Lathe, G H. (1953) "The separation of serum pigments giving the direct and indirect van den Bergh reaction", *J clin Path*, **6**, 99
Cole, P G., Lathe, G H, and Billing, B H (1954) "Separation of the bile pigments of serum, bile and urine", *Biochem J*, **57**, 514
Comline, R S, Roberts, H E, and Titchen, D A. (1951). "Route of absorption of colostrum globulin in the newborn animal", *Nature*, (*Lond*), **167**, 561
Coombs, R. R A, Crowhurst, R C, Day, F T, Heard, D H, Hinde, I. T., Hoogstraten, J, and Parry, H. B. (1948) "Haemolytic disease of newborn foals due to iso-immunization of pregnancy", *J. Hyg*, *Camb*, **46**, 403

Coombs, R. R. A., Gorius, J., and Bessis, M. (1950). "Diagnostic de l'ictère hémolytique des muletons par le test à l'antiglobuline", *C. R. Soc. Biol.*, 144, 688.

Coombs, R. R. A., Gleeson-White, M. H., and Hall, J. G.

Coombs, R. R. A., and Mourant, A. E. (1947) "On certain properties of antisera prepared against human serum and its various protein fractions, their use in detection of sensitisation of human red cells with 'incomplete' Rh antibody, and on nature of this antibody", *J. Path. Bat.*, 59, 105.

Coombs, R. R. A., Race, R. R., and Mourant, A. E. (1945 *a*) "Detection of weak and 'incomplete' Rh agglutinins; a new test", *Lancet*, ii, 15.

8, 620

Crawford, H., and Mollison, P. L. (1951) "Demonstration of multiple antibodies in antiglobulin sera", *Lancet*, ii, 955

Cronin, M. T. I. (1953). "Exchange transfusion in the foal", *Vet. Rec.*, 65, 120.

Cronin, M. T. I. (1955) "Haemolytic disease of newborn foals", *Vet. Rec.*, 67, 479.

Crosse, V. M., Meyer, T. C., and Gerrard, J. W. (1955). "Kernicterus and Prematurity", *Arch. Dis. Childh.*, 30, 501.

Cserna, S., and Liebmann, S. (1923). "Beitrag zur Lehre des Icterus Neonatorum", *Klin. Wschr.*, **2**, 2122.

Culbertson, C. G. (1938). "Natural transmission of immunity against trypanosoma lewisi from mother rats to their off-spring", *J. Parasit.*, **24**, 65.

Culbertson, C. G. (1939) "The immunization of rats of different age groups against trypanosoma lewisi by the administration of specific antiserum per os", *J. Parasit.*, **25**, 181.

Culbertson, C. G. (1940). "The natural transmission of immunity against trypanosoma duttoni from mother mice to their young", *J. Immunol.*, **38**, 51.

Culbertson, C G., and Ratcliffe, A. W. (1936). "Reaction following intra-group blood transfusion: irregular agglutinin demonstrated by the sensitive centrifuge test method", *Amer. J med. Sci.*, **192**, 471.

Cutbush, M., Crawford, H., and Mollison, P. L. (1955) "Observations on anti-human globulin sera", *Brit. J. Haemat.*, **1**, 410.

Cutbush, M, Giblett, E. R., and Mollison, P. L. (1956). "Demonstration of the phenotype Le(A+B+) in infants and adults", *Brit. J. Haemat.*, **2**, 210

Dacie, J. V. (1951) "Differences in the behaviour of sensitised red cells to agglutination by antiglobulin sera", *Lancet*, ii, 954

Dacie, J V. (1954) "The Haemolytic Anaemias, Congenital and Acquired" Churchill, London

Dahr, P (1947). "Medizinischen Gesellschaft." Gottingen.

Dameshek, W, and Schwartz, S O (1938) "Haemolysis as the cause of clinical and experimental hemolytic anaemics", *Amer. Jour Med Sci*, **196**, 769

Darrow, R. R. (1938). "Icterus gravis (erythroblastosis) neonatorum", *Arch. Path*, **25**, 378

Darrow, R. R, and Chapin, J (1947). "Pathogenesis of passive Rh isosensitization in the newborn (erythroblastosis fetalis)", *Amer Jour. Dis. Child.*, **73**, 257

Davies, B S., Gerrard, J, and Waterhouse, J. A. H. (1953) "The pattern of haemolytic disease of the newborn", *Arch Dis. Childh*, **28**, 466.

Davidson, L. T., Merritt, K. K., and Weech, A A. (1941). "Hyperbilirubinemia in the newborn", *Amer Jour. Dis Child*, **61**, 958

Day, R (1947). "The Kernicterus problem experimental in vivo and in vitro staining of brain tissue with bilirubin", *Amer. Jour Dis. Child.*, **73**, 241.

[illegible]

haematin and mesobilirubin. Discussion. *Amer. Jour. Dis. Child.*, 90, 643.

Day, R. L. (1956) "Kernicterus", *Pediatrics*, Springfield, 17, 920.

[illegible]

De[illegible]

De[illegible]

Folge), 273.

Dereymaeker, A. (1949) "Contribution à l'étude clinique, [illegible]

627

Dewes, W. P. (1826). "A Treatise on the Physical and Medical Treatment of Children" p 303 London

Diamond, L K., Blackfan, R. O, and Baty, J. H. (1932) [illegible]

Dimock, W W., Edwards, P R, and Bruner, D. W. (1947) "Infections observed in equine fetuses and foals", *Cornell Vet*, 37, 89.

Dodd, M C, Wright, C-S, Blaxter, J A, Bouroncle, B A., Brunner, A. E, and Winn, H. J. (1953) "The immunologic specificity of antiserum for trypsin-treated red blood cells and its reactions with normal and haemolytic anaemic cells", *Blood*, 8, 640.

Doglioni, V. (1936). "[illegible] materno e [illegible] rista della [illegible] heoretico- [illegible] ff und die [illegible] *und Phys. und f. klin. Med.* [illegible]

[illegible] "Isohaemolytic disease of [illegible] 5.

[illegible] bservations on haemolytic [illegible] *Vet*, 41, 14.

[illegible], M. E., and Bryans, J. T. [illegible] ie fetal tissue vaccine on haemagglutination activity of mare serums: its relation to hemolytic icterus of newborn foals", *Cornell Vet.*, 42, 495.

Dollet, M (1939). "L'ictère nucleaire der nouveau-né." Thèse de Lyons.

Donatien, A., Lestoguard, F., and Sausseau, L. (1924). "Piroplasmes trouvés dans un cas de jaunisse du muleton du Poitou", *C. R. Soc Biol.*, 90, 1308. See also "Piroplasmes et [illegible]

D[illegible] m [illegible]",

Dublin, W. B. (1949). "Pathogenesis of Kernicterus", *Jour. Neuropath and Exp Neurol.*, 8, 119

Dublin, W. B (1951). "Neurologic lesions of erythroblastosis foetalis in relation to nuclear deafness", *Am. J. Clin. Path*, 21, 935.

Dummelius, J. (1769). "De ictero neonatorum." Basle.

Duges, A. (1832). *Dict. de Méd et de Chir. Prat.*, 8, 301.

Duguid, W. R (1906) "A series of cases of icterus neonatorum", *Brit Med Jour*, i, 319

Duttel, P J (1702) "De morbis foetuum in utero materno." Halae Magdeburgicae

Edgecombe, K. (1930). "Isohaemagglutinins the influence of the foetus upon the titre of the mother's blood during pregnancy", *J Path Bact*, 33, 963.

Ehrlich, P. (1892) "Ueber Immunitat durch Vererbung und S[illegible]", *Z. Hyg. Infekt.*, [illegible]

Chilan, 28, 403.
Engel, M. (1940) "Quantitative Untersuchungen zur Gallen-
(*Hoppe-*
927). "Die
auf den
ein gelbes
ademiae Caesareo-
iae III, Annus II.
Fe with jaundice and edema in the newly born", *Amer. J Path*, 7, 277
Findlay, L. (1946). "The blood in infancy", *Arch Dis. Childh*, **21**, 195
Findlay, L, Higgins, G, and Stanier, M W. (1947) "Icterus
on mortality in haemolytic disease of the newborn", *Brit Med J*, **i**, 615
Fisk, R T, and McGee, C A (1947) "The use of gelatin in Rh testing and antibody determination", *Amer J clin Path*, **17**, 737
Flamens (1840) Cited by Lhomme, 1867 Also by Terré, G (1948) "L'isoimmunisation et l'ictere grave des muletons" Thesis Toulouse
Fordyce, A D, and McAfie, W G (1924) "The tendency of the new-born to jaundice, with report of a case of grave familial jaundice", *Lancet*, **i**, 1151.

Forrester, R. M., and Miller, J. (1955). "The dental changes associated with kernicterus", *Arch. Dis. Childh.*, 30, 224

Forster, F. M., and McCormack, R. A. (1944) "Kernicterus unassociated with Erythroblastosis Fetalis", *J. Neuropath and Exp. Neurol.*, 3, 379.

Frank, J. (1843). "Praexeos Medicae Universae Praecepta", Par. III, Vol. II, Sect. II, fasc. I, para. 54, pp. 302. 305. Leipzig.

Fuchs, A. (1914). Uber *einen experimentell-toxischen, choreiformen Symptomenkomplex beim Tiere*", *Jahrb. f. Psychiat. u. Neurol.*, 36, 165.

Gairdner, D, Marks, J., and Roscoe, J. D (1952). "Blood formation in infancy. I. The normal bone marrow; II. Normal erythropoiesis", *Arch. Dis. Childh.*, *27*, *128* and *214*

Gairdner, D., Marks, J., and Roscoe, J. D. (1955) "Blood formation in infancy. IV The early anaemia of prematurity", *Arch. Dis Childh.*, *30*, *203*.

Gardien, M. (1824) "Traité complet d'Accouchemens", Volume 4, p. 83, 3rd edition, Paris.

Gauchot, G. (1936). "*De l'étiologie et du traitement de l'ictère* infectieux des muletons." Thesis. Lyons.

Gellé, P. (1803) "Sur l'ictère des muletons nouveau-nés", Mém. de la Soc. Agric. des Deux Sèvres.

Gerrard, J. (1952). "Kernicterus", *Brain*, 75, 526

Giblett, E R, Varela, J E., and Finch, C A. (1956). "Damage of the bone marrow due to Rh antibody", *Pediatrics*, Springfield, 17, 37.

Gilmour, J. R (1944). "Erythroblastosis foetalis", *Arch. Dis. Childh*, 19, 1, 12 and 21.

Glaister, J. (1879). "Icterus neonatorum. Notes of a case in which a *congenital stricture of the ductus communis choledochus* was found on post-mortem examination", *Lancet*, I, 293 and 331.

Gleeson-White, M. H, *Heard, D. H*, *Mynors, L. S*, and Coombs, R R. A (1950) "Factors influencing the agglutinability of red cells the demonstration of a variation in the susceptibility to agglutination exhibited by the red cells of individual oxen", *Brit J. exp , Path* , 31, 321.

Goldsmith, K. (1955) "*Papain-treated red cells in the detection* of incomplete antibodies", *Lancet*, i, 76

Goodhart, J. F. (1883) "Atlas of illustrations of Pathology." *Vol I, fasc. 5, p* 2, "A résumé of diseases of the liver, gall-bladder and larger bile-ducts" New Sydenham Society, London.

Goodwin, R. F. W. (1955) "Some common factors in the

317.

Goodwin, R. F. W., Hayward, B. H. G., Heard, D. H, and Roberts, G. Fulton (1955) "Acquired haemolytic anaemia occurring in newborn piglets", *Sang*., 26, 24.

Goodwin, R. F. W., Heard, D. H., Hayward B H. G., and Roberts, G. Fulton (1956). "Haemolytic disease of the new-

J. comp. Path, 65, 79.

"The blood groups of the epidemiology of *J. comp. Path.*, 67,

Govan, A. D T., and Scott, J M (1953) "Kernicterus and prematurity", *Lancet*, I, 611.

Grandidier, L (1871) "Der freiwilligen Nabelblutungen der

Gurevitch, J, Polishuk, Z, and Hermoni, D (1947). "The role of a presumed serum protein in the pathogenesis of erythroblastosis fetalis", *Amer. J. clin. Path.*, 17, 465.

Hagendorn, D. E. (1698). "Observationum et historiarum medico-practicarum rariorum", *Centuriae Tres, Historia 57*, p. 356. Frankfurt.
Halban, J. (1900). "Agglutinationsversuche mit mutterlichem und kindlichen Blute", *Wien klin. Woch.*, 13, 545.
Halbrecht, I. (1951). "Icterus Precox; further studies on its frequency, etiology, prognosis and the blood chemistry of the cord blood", *J. Pediat.*, 39, 185.
Halliday, R (1955 *a*). "The absorption of antibodies from immune sera by the gut of the young rat", *Proc. Roy. Soc. B.*, 143, 408
Halliday, R (1955 *b*). "Prenatal and postnatal transmission of passive immunity to young rats", *Proc. Roy. Soc. B*, 144, 427.
Halliday, R. (1956). "The termination of the capacity of young rats to absorb antibody from the milk", *Proc. Roy. Soc. B.*, 145, 179.
Hanon, F., Coquoin-Carnot, M., and Pignard, P. (1955) "La Liquide Amniotique." Masson, Paris.
Hansen, R. G, and Phillips, P H (1947). "Studies on proteins from bovine colostrum. I. Electrophoretic studies on the blood serum proteins of colostrum-free calves and of calves fed colostrum at various ages", *J. biol Chem*, 171, 223.
Hartley, P (1951) "The effect of peptic digestion on the properties of diphtheria antitoxin", *Proc. Roy. Soc. B*, 138, 499.
Hartmann, A (1880). "Monatsschrift d. ver. d Thier in Oest." *Detailed summary given by M G. Neumann (1881), Rév. Vét*, 6, 133
Hartmann, H. (1928) "Die allgemeine angeborene Wassersucht von Frucht und Placenta", *Zentralb f. Gynäk*, 52, 299.
Hatfield, M P (1889) "Five fatal cases of biliary cirrhosis (congenital pernicious icterus) in the same family", *Trans Amer Pediat, Soc.*, 1, 147.
Heard, D. H. (1955 *a*) "The recognition of four red cell antigen-antibody systems in the rabbit", *J. Hyg, Camb*, 53, 398
Heard, D H (1955 *b*) "Factors influencing the agglutinability of red cells *Variation of the red cells of the rabbit in susceptibility to agglutination by homologous iso-antisera*", *J Hyg, Camb.*, 53, 408
Heard, D H, Hinde, I T., and Mynors, L S (1949). "An experimental study of haemolytic disease of the newborn due to isoimmunization of pregnancy I An attempt to produce the syndrome in the rabbit", *J. Hyg, Camb.*, 47, 119.
Hedenstedt, S, and Vahlquist, B. (1948) "The erythrocyte turnover during the neonatal period. Experiments with elliptocyte transfusions to newborns", *Acta Paediat.*, 35, 355

Hervieux, J. F. E. (1847). "De l'ictère chez les nouveau-nés." Thesis Paris.
Herz, M. (1878). "Zur Casuistik der acuten Fettenartung bei Neugebornen", *Oesterr. Jahrb. f. Paediat*, 8, 139.
Hess, A. (1953). "Relation of the ground substance of the central nervous system to the blood-brain barrier", *Nature (Lond.)*, 175, 387.
Hill, J. M., and Haberman, S. (1954). "The Coombs (antiglobulin) test: Indications and technics", *Amer. J. clin. Path.*, 24, 305.
Hirszeld, L., and Zbrowski, H. (1925). "Sur la perméabilité [illegible]
[illegible]
500.
Homan, J. (1849) "Cases of umbilical haemorrhage in infants", *Boston, Med and Surg. J.*, 40, 449.
Hoogeveen, T. (1784). "Tractatus de Foetus Humani morbis." Lugduni Batavorum, cap. 5, p 59.
Horst, J. O. (1673). "Exhibens casum de foetu abortivo icterico" Dissertation. Giessen.
Howard, F. A., and Cronin, M T. I. (1955). "Colostral transfer of anti-erythrocyte agglutinins from mare to foal", *Amer J Vet. Med Ass*, 126, 93.
Howe, P. E. (1921) "An effect of the ingestion of colostrum on the composition of the blood of new-born calves", *J. biol Chem.*, 49, 115.
Hoyt, R. E., and Zwicker, H. (1953). "The role of enzyme in reversible agglutination of red cells", *J Immunol.*, 71, 325.
Hsia, D. Y., Allen, F H., Diamond, L. K., and Gellis, S S (1953) "Serum bilirubin levels in the newborn infant", *J. Pediat*, 42, 277.
Hubbard, J. C. (1913). "An unusual obstetrical history", *Boston Med. and Surg. J.*, 168, 459
Hutchinson, R. (1904) "Some disorders of the blood and [illegible] 53.
[illegible] a placenta-
[illegible] H. (1942).
[illegible], *Proc. Soc. exp. Biol.*, 51, 103.

Jandl, J. H., and Castle, H. B. (1956). "Agglutination of sensitized red cells by large anisometric molecules", *J. Lab. clin. Med.*, 47, 669.

Jenkins, J. F. (1858). "Report on spontaneous umbilical haemorrhage of the newly-born", *Trans. Amer. Med Ass.*, **11**, 261.

Johnson, R. A., and Conway, J. F. (1933). "Urinary suppression and uremia following transfusion of blood", *Amer. J. Obstet. Gynec.*, 26, 255.

Johnstone, J. M. (1953). "Bilirubin values of cord blood in heter[illegible]

Jones, A [illegible]
lent a [illegible]

Jonsson, [illegible]
gersch [illegible]

Joysey, [illegible]
with r [illegible]
agglut [illegible]
32, 440.

Juncker, D. L. (1724). "Conspectus Medicinae theoretico-[illegible]

[illegible]
26, 311

Keeler, C. E., and Castle, W. E (1933) "A further study of blood groups of the rabbit", *Proc. Nat. Acad. Sci*, **19**, 403

Keeler, C. E., and Castle, W. E. (1934 *a*). "Blood group incompatibility in rabbit embryos and in man", *Proc. Nat. Acad. Sci.*, 20, 273

Keeler, C. E, and Castle, W. E (1934 *b*). "The influence of [illegible]

Kelsall, G. A., and Vos, G. H. (1955). "Premature induction of labour in the treatment of haemolytic disease of the newborn", *Lancet*, ii, 161.

Kerckring, T. (1670). "Spicilegium Anatomicum", obs 57, p 118. Amsterdam

Kershaw, G F. (1950). "Death of young piglets", *Vet. Rec*, 62, 383.

King, W. W. (1908). "Generalised oedema of the foetus", *Lancet*, ii, 532.

Kirschbaum, W. (1922). "Uber den Einflus schwerer Leber- [illegible]

[illegible] by papain for detection of Rh antibodies", *Amer. J. clin. Path*, 20, 1067.

Küster, F., and Krings, H. (1950). "Blood destruction and cerebral damage in haemolytic diseases of the newborn." [illegible]

Landsteiner, K., and W[illegible] (1940) [illegible]

La[illegible] *acta paediat*, Stockh, 31, 133

Lathe, G. H. (1951) [illegible] ha[illegible]

Lath[illegible] 1[illegible] No 12, p 34

Lathe, G H (1955) "Exchange transfusion as a means of removing bilirubin in haemolytic disease of the newborn", *Brit med J*, i, 192

Lathe, G H (1956) "Bilirubin" Correspondence *Lancet*, ii, 683.

Laurance, B (1955) "Danger of Vitamin-K analogues to Newborn" Correspondence. *Lancet*, i, 819.

Leger, T (1923) "Considération sur l'endurcissement du tissu cellulaire chez les nouveau-nés." Thesis Paris.

Lemberg, R., and Legge, J. W. (1942). "Intracorpuscular bile pigment formation", *Aust. J. exp. Biol med Sci*, **20**, 65
Lemberg, R., and Legge, J. W. (1949). "Haematin compounds and bile pigments." Interscience, London and New York.
Lenart, G. (1928) "Icterus neonatorum eine Folge von Isoagglutinationserscheinungen", *Jahrb. f. Kinderheilk.*, **121**, (B 71, III Folge), 134.
Lenart, G., and Biro, S. (1929). "Die Isoagglutination bei den Neugeborenen und ihre Beziehungen zum Icterus neonatorum", *Jahrb f. Kinderheilk.*, **124** (B. 74, III Folge), 77.
Leuret, P. (1906). "Remarques sur la pathogénie de l'ictere des nouveau-nés; phenoménes d'hematolyse", *Folia haemat., Lpz.*, **3**, 81.
Levine, P. (1946). "The present status of the Rh factor", *Amer. J clin Path.*, **16**, 597.
Levine, P. (1955). "Haemolytic disease due to antibodies other than anti-D", *Rev d'Hémat.*, **10**, 215.
Levine, P, Burnham, L., Katzin, E. M., and Vogel, P. (1941) "The role of isoimmunization in the pathogenesis of erythroblastosis fetalis", *Amer. J Obstet. Gynec.*, **42**, 925.
Levine, P, and Katzin, E. M. (1940). "Iso-immunization in pregnancy and the varieties of isoagglutinins involved", *Proc Soc. exp. Biol.*, **45**, 343.
Levine, P, and Stetson, R. E. (1939) "An unusual case of intragroup agglutination", *J Amer Med. Ass*, **113**, 126
Levine, P, Vogel, P, and Rosenfield, R E (1953). "Haemolytic disease of the newborn", *Advanc Pediat.*, **6**, 97.
Levrier (1850). "Quelques notes sur les maladies des poulain set des muletons", *Rec méd vét pract*, **27**, 973.
Lewis, M, and Chown, B. (1954) "Hidden anti-Rhesus saline antibodies", *Nature (Lond)*, **173**, 44
Lhomme (1867). "L'hematurie des jeunes muletons pendant le premier âge", *Jour. des vét du midi*, **30**, 221.
Liebling, J, and Schmitz, H. E (1943). "Colostrum as source of diphtheria antitoxin in actively immunized pregnant mothers", *J. Pediat*, **22**, 189
Lin, H, and Eastman, N. J (1937). "Behaviour of intravenously injected bilirubin in newborn infants", *Amer J Obstet. Gynec*, **33**, 317.
Litchfield, H R (1945) "Erythroblastosis fetalis", *J. Pediat.*, **27**, 353
Little, R. B, and Orcutt, M L (1922) "The transmission of agglutinins of *Bacillus abortus* from cow to calf in the colostrum", *J. exp. Med.*, **35**, 161.

Lobstein, J. F. (1826). "Memoire sur la kirronose", *Répertoire générale d'anatomie et de Physiol Path. et de clin. Chir.*, 1, 28.

Lochead, J. (1901). "On the transmission of nitrogenous compounds from mother to foetus", *Trans. Edinb. Obstet. Soc.*, 33, 120.

London, I M (1950). "The conversion of hematin to bile pigment", *J. biol. Chem.*, 184, 373

London, I. M , and West, R (1950). "The formation of bile pigment in pernicious anaemia", *J. biol Chem* , 184, 359.

Longsworth, L. G , Curtis, R. M., and Pembroke, R. H. (1945). "The electrophoretic analysis of maternal and fetal plasmas and sera", *J. clin Invest.*, 24, 46

Marks, J , Gairdner, D. M. T., and Roscoe, J. D. (1955). "Blood formation in infancy. III. Cord blood", *Arch. Dis. Child* , 30, 117.

MacLean, J. R., Lucy, J. F , and Harris, R C (1955) "Study of bilirubinemia of prematures with relation to kernicterus", *Amer J. Dis Child.*, 90, 573

McCulloch, E A. (1950). "Demonstration of incomplete Rh antibody by alpha globulin", *Nature (Lond.)*, 165, 276

McDuffie, F C , and Kabat, E A. Cited by Kabat (1956), p 264.

M'Gibbon, J. (1913) "Fatal cases of jaundice in the newborn child with notes of a case in successive pregnancies", *Trans. Edinb Obst. Soc* , 38, 285

McGirr, J. L. (1947) "Colostral transmission of antibody substances from mother to offspring", *Vet Jour* , 103, 345

McQuarrie, I (1923) "Isoagglutination in new-born infants and their mothers", *Bull Johns Hopkins Hosp* , 34, 51

Makinodan, T , and Macris, N T (1955) "The effect of ficin on the agglutination of human red blood cells", *J Immunol* , 75, 192.

Mason, J H , Dalling, T , and Gordon, W. S. (1930) "Transmission of maternal immunity", *J Path. Bact* , 33, 783

Maubaret, J P. (1932) "La jaunisse des muletons," Thesis, Alfort.

Medico-Chirurgical Review (1834) "Haemorrhagic tendency in a family" (an abstract from *Jour Pract Heilk*), 21, 232

Meldolesi, G , Siedel, W , and Moller, H (1939) "Ueber Myobilin", *Zeitschr f physiol chem. (Hoppe-Seyle)*, 259, 137

Mella, H (1924) "The experimental production of basal ganglion symptomatology in *Macacus rhesus*", *Arch. Neurol. and Psychiat* , 11, 405

Mandelbaum, H (1939) "Hemolytic reaction following blood transfusion report of a case of intra-group incompatibility", *Ann Int Med* , 12, 1699

Meyer, T. C., and Angus, J. (1956). "The effect of large doses of synkavit in the newborn", *Arch. Dis. Childh*, **31**, 212.

Michael, J. (1688). Observat. praexeos clinicae Spec. cas XXIII, 557.

Miller, H. C., Johnson, R. D., and Durlacher, S. H. (1944). "Comparison of newborn infants with erythroblastosis fetalis with those born to diabetic mothers", *J. Pediat.*, **24**, 603.

Millot, P., and Gorius, J. (1950). "Considérations sur la physiopathologie de l'ictère hémolytique de muleton; rôle de l'allaitement; importance de colostrum", *Rev. Path. Comp*, **50**, 85.

Mitchell, J. M. (1928). "The role of haemolysis in jaundice of the new-born infant", *Amer. J. Dis. Child.*, **36**, 486.

Mollison, P. L. (1948). "Physiological jaundice of the newborn, some new measurements of the factors concerned", *Lancet*, **i**, 513.

Mollison, P. L. (1956 *a*). "Blood transfusion in Clinical Medicine" Second edition. Blackwell, Oxford.

Mollison, P. L. (1956 *b*). Paper read at first meeting of British Society for Immunology, later published by Hughes Jones, N. C, Mollison, P L., and Veall, N. (1957) "Removal of incompatible red cells by the spleen", *Brit. J Haemat.*, **3**, 125.

Mollison, P. L., Boorman, K. E, and Dodd, B. E. (1944). "Incidence of haemolytic disease of the foetus ('erythroblastosis fetalis') in different familes. Value of serological tests in diagnosis and prognosis", *J. Obstet. Gynaec., Brit. Emp*, **51**, 1.

Mollison, P L., and Cutbush, M (1951) "A method of measuring the severity of a series of cases of haemolytic disease of the newborn", *Blood*, **6**, 777.

Mollison, P L., and Cutbush, M. (1954) "Recent Advances in Paediatrics", ed. Gairdner, D M T. Churchill, London, p 110.

Mollison, P. L, and Cutbush, M (1955) "Use of isotope-labelled red cells to demonstrate incompatibility in vivo", *Lancet*, **i**, 1290

Mollison, P. L, Veall, N., and Cutbush, M (1950). "Red cell and plasma volume in newborn infants", *Arch. Dis. Childh*, **25**, 242.

Mollison, P. L, and Walker, W. (1952). "Controlled trials of the treatment of haemolytic disease of the newborn", *Lancet*, **i**, 429.

Morgagni, G. B. (1761). "De sedibus et caussis morborum", Lib. III, Epist, 48, Art 60 Lugduni Batavorum.

Morison, J. Edgar (1952). "Foetal and Neonatal Pathology." Butterworth, London.

Morton, J. A., and Pickles, M. M. (1947). "Use of trypsin in the detection of incomplete anti-Rh antibodies", *Nature (Lond.)*, **159**, 779.

Morton, J. A., and Pickles, M. M. (1951). "The proteolytic enzyme test for detecting incomplete antibodies", *J. Clin. Path.*, **4**, 189.

Mossu, R., Giradeau, and Maubaret, J. P. (1924). "Recherches sur la nature et le traitement de la jaunisse des muletons nouveau-nés", *C.R. Soc. Biol.*, **91**, 68.

Muller, J. F. (1788). "Origines icteri maxime eius qui infantes recens natos occupat."

Munk-Andersen, G. (1956). "A dextran serum medium for the demonstration of incomplete anti-A and anti-B", *Acta path. microbiol. scand.*, **38**, 259.

Murray, H. L. (1910). "The hemotoxic nature of eclampsia with an account of the foetal and placental haemolysins and an experimental investigation into the anaphylactic theory of eclampsia", *J. Obst. Gyn. Brit. Emp.*, **18**, 225.

[illegible]

guinea-pigs", *J. Hyg., Camb.*, **48**, 456.

Nachtsheim, H., and Klein, H. (1947). "Hydrops congenitus universalis beim Kaninchen, eine Erbliche fetale erythroblastose" Abhandlungen der Deutschen Acadamie der Wissenschaften zu Berlin, No. 5, 1.

Needham, J. (1931). "Chemical Embryology" Cambridge. Vol. III, p. 1510 et seq.

[illegible]

J. Amer. vet. med. Ass., **129**, 301.

Meyer, T. C., and Angus, J. (1956). "The effect of large doses of synkavit in the newborn", *Arch. Dis. Childh.*, **31**, 212.

Michael, J. (1688). Observat. praexeos clinicae Spec. cas. XXIII, 557.

Miller, H. C., Johnson, R. D., and Durlacher, S. H. (1944). "Comparison of newborn infants with erythroblastosis fetalis with those born to diabetic mothers", *J. Pediat.*, **24**, 603.

Millot, P., and Gorius, J. (1950). "Considérations sur la physiopathologie de l'ictère hémolytique de nuleton; rôle de l'allaitement; importance de colostrum", *Rev. Path. Comp.*, **50**, 85.

Mitchell, J. M. (1928). "The role of haemolysis in jaundice of the new-born infant", *Amer. J. Dis. Child.*, **36**, 486.

Mollison, P. L. (1948) "Physiological jaundice of the newborn, some new measurements of the factors concerned", *Lancet*, **i**, 513.

Mollison, P. L. (1956 *a*). "Blood transfusion in Clinical Medicine." Second edition Blackwell, Oxford.

Mollison, P. L (1956 *b*). Paper read at first meeting of British Society for Immunology, later published by Hughes Jones, N C, Mollison, P. L., and Veall, N. (1957). "Removal of incompatible red cells by the spleen", *Brit. J. Haemat.*, **3**, 125.

Mollison, P. L., Boorman, K. E., and Dodd, B. E (1944). "Incidence of haemolytic disease of the foetus ('erythroblastosis fetalis') in different famiiles Value of serological tests in diagnosis and prognosis", *J. Obstet Gynaec., Brit. Emp.*, **51**, 1.

Mollison, P. L., and Cutbush, M. (1951). "A method of measuring the severity of a series of cases of haemolytic disease of the newborn", *Blood*, **6**, 777.

Mollison, P L, and Cutbush, M. (1954) "Recent Advances in Paediatrics", ed Gairdner, D M T Churchill, London, p. 110.

Mollison, P. L, and Cutbush, M. (1955) "Use of isotope-labelled red cells to demonstrate incompatibility in vivo", *Lancet*, **i**, 1290

Mollison, P. L, Veall, N, and Cutbush, M (1950) "Red cell and plasma volume in newborn infants", *Arch Dis. Childh.*, **25**, 242.

Mollison, P. L., and Walker, W. (1952). "Controlled trials of the treatment of haemolytic disease of the newborn", *Lancet*, **i**, 429

Morgagni, G. B. (1761) "De sedibus et causis morborum", Lib. III, Epist, 48, Art. 60. Lugduni Batavorum

Morison, J. Edgar (1952). "Foetal and Neonatal Pathology." Butterworth, London

Pentschew, A. (1948). "Genesis of encephalopathia post-

153, 880.

Pickles, M. M. (1949). "Haemolytic disease of the newborn" Blackwell, Oxford, p. 111.

Pigoury, L., and Charney, J. (1950) "Reproduction expérimentale de l'ictère hémolytique du muleton", *Bull. Acad vét. Fr.*, **24**, 399.

152, 413

Potter, E L (1947) "Rh: its relation to congenital haemolytic disease and to intragroup reaction." The Year Book publishers, inc, p 237

Pout, G (1822) "A case of umbilical haemorrhage which ... **12**, 183.

... in a young foal",

... es muletons", *Bull.* ... *vét*, **10**, 552

... de muleton", *Rec.*

... tons nouveau-nés", *Rev Med vet (Lyons et Toulouse)*, 99, 503.

Race, R R (1944) "An 'incomplete' antibody in human serum", *Nature (Lond)*, **153**, 771.

Race, R R, and Sanger, R (1954). "Blood groups in Man." Second Edition Blackwell, Oxford.

Nicloux, M. (1909). *L'Obstétrique*, 11, 840.
Nicolajev, V. (1937). "Zur Frage der Beziehungen zwische Leber und Gehirn", *Arch f. path. Anat. u. Phys u. f. kli Med.* (*Virchow's Archives*), 299, 309.
Oberndorfer, S. (1927). "Hydrops congenitus universalis" *Zbl. f. Gynäk.*, 51, 1830.
Obrinsky, W., Allen, E. L., and Anderson, E. E. (1954). "Physio logic hyperbilirubinema in premature infants", *Amer. J. Di Child.*, 87, 305.
Obrinsky, W., Denley, M. L., and Brauer, R. W. (1952). "Sulfo bromophthalein sodium excretion test as a measure of live function in premature infants", *Pediatrics, Springfield*, 9, 421
Opitz, E. (1903). "Zur Biochemie der Schwangerschaft", *Dtsch med Wschr.*, 29, 597.
Orth, J. (1875). "Ueber das Vorkommen von Bilirubinkrystalle bei neugebornen Kindern", *Arch. f. path Anat. Phys. u. f klin. Med.* (*Virchows Arch.*), 63, 447.
Ottenberg, R. (1923). "The etiology of eclampsia", *J. Amer. med Ass*, 81, 295.
Otto, A W. (1830). "Lehrbuch der pathologischen anatomie de menschen und der Thiere", footnote 2 on p 37 of South' English translation.
Owen, R. D. (1956). "Immunological tolerance." Discussion a the Royal Society reported in *Brit. med. J.*, I, 740; *Proc. Roy Soc B*, 146, 8.
Owen, R D, Wood, H. R, Foord, A G, Sturgeon, P., and Baldwin, L G. (1954). "Evidence for actively acquired toler ance to Rh antigens", *Proc Nat. Acad Sci. Wash.*, 40, 420
Panaroli, D. (1654) "Iatrologismorum sive observationum medicinalium." Pentecostae quarta, obs 44, p. 137 Hanover.
Parr, L W, and Krischner, H. (1932). "Haemolytic transfusion fatality with donor and recipient in the same blood group", *Jour Amer Med Ass.*, 98, 47
Parry, H B, Day, F. T., and Crowhurst, R C (1949). "Diseases of newborn foals I Haemolytic disease due to iso-immunisation of pregnancy", *Vet. Rec*, 61, 435
Parsons, L. G. (1947). "The clinician and the Rh factor", *Lancet*, I, 815.
Pasachoff, H. D., and Wilson, L. (1931) "Congenital anemia of the new-born", *Amer. J Dis Child*, 42, 111.
Pearson, quoted in Underwood, M. (1799).
Pedersen, K. O. (1944) "Fetuin, a new globulin isolated from serum", *Nature (Lond)*, 154, 575.
Pennavaria and Baglieri (1865). Cited by Cadeac, 1896

Ryan, M. (1835). "Lectures on the physical education and diseases of infants from birth to puberty. No 36", *London Med. Surg. J.*, 7, 517.

Sa

Sa

, *Rev. méd vét*, 10, 468.

s muletons en Poitou",

Sausseau, L. (1925). "L'Ane, les chevaux mulassiers et la mule de Poitou". Paris, p 291

Sborov, V. M , Jay, A. R , and Watson, C. J. (1949) "The effect of aureomycin on urobilinogen formation and the fecal flora", *J. Lab clin Med*, 34, 1743

Schmorl, G (1903). "Zur Kenntnis des Ikterus neonatorum, insbesondere der dabei auftretenden Gehirnveranderungen", *Verhandl d. Deut Path Gesell*, 6, 109 (Paper read in 1903; proceedings published in 1904)

Schneider, L , and Szathmary, J (1938) "Uber die immunität der neugeborenen saugetiere", *Z ImmunForsch*, 94, 458, 465 and 95, 465

Sc

Schulman, I , Smith, C. H., and Stern, G S (1954) "Studies on the anemia of prematurity", *Amer J Dis. Child*, 88, 567.

Schultze, G (1676) "De Manna Abortus Causa", obs. 241 on p 353 in

naturae cu

Germanica

Schurig, M

and Leipzig

Seifart, O (1899) Gerhardt's "Lehrbuch der Kinderkrankheiten " Tubingen.

Race, R. R., Sanger, R., and Lehane, D. (1953) "Quantitative aspects of the blood-group antigen Fy^a", *Ann. Eugen., Lond.*, **17**, 255.

Race, R. R., Sanger, R., and Selwyn, J. G. (1951) "A possible deletion in a human Rh chromosome; a serological and genetical study", *Brit. J. exp. Path.*, **32**, 124.

Rainard (1867). Cited by Lhomme, 1867.

Ranström, S. (1951). "The morbid anatomy of erythroblastosis fetalis and its relation to the Rh-factor", *Acta paediat., Uppsala*, **40**, 41.

Ratner, B., Jackson, H. C., and Gruehl, H. L. (1927). "Transmission of protein hypersensitiveness from mother to offspring", *J. Immunol.*, **14**, 249

Rautmann (1912). "Ueber angeborene Wassersucht", *Munch. med. Wschr.*, **59**, 2590

Ray, E. (1849) "On haemorrhage from the umbilicus after the separation of the funis", *Med. Gaz.*, N.S., **8**, 423.

Reisner, E. H., Jr. (1943) "Morphology of erythrocytes and erythroblastosis foetalis", *Arch. intern. Med.*, **71**, 230.

Reynal, see Bouley and Reynal.

Rimington, C., and Stewart, A. M. (1932). "A pigment present in the sweat and urine of certain sheep. Its isolation properties and relationship to bilirubin and the metabolism of haemoglobin generally", *Proc. Roy. Soc. B*, **110**, 75.

Ritchie (1912) Cited in M'Gibbon (1912) Discussion

Roberts, G. Fulton (1950) "The antiglobulin reaction", Proc. 3rd Int. Cong. of Haematology, p. 147.

Rolleston, H. D. (1910) "Remarks on a case of recurring jaundice in four successive pregnancies with fatal jaundice in three successive infants", *Brit. med. J.*, **i**, 864.

Rolleston, H. D., and McNee, J. W. (1929) "Diseases of the Liver, Gall-bladder and Bile ducts" Third edition Macmillan, London, p. 610

Romagnoli, A., Del Bono, G., and Parri, O. (1956). "Malattia emolitica in suinetti neonati", *Atti Soc. ital. Sci. vet.*, **9**, 596.

Rosenthal, M. C., and Schwartz, L. I. (1951) "Reversible agglutination of trypsin treated erythrocytes by normal human sera", *Proc. Soc. exp. Biol.*, **76**, 635.

Rothe-Mayer, A., and Hickmans, E. M. (1952) "Decrease of serum cholesterol ester in haemolytic disease of the newborn", *Arch. Dis. Child.*, **20**, 160

Royer, M., and Bertrand, J. C. (1929). "L'urobiline chez le nouveau-né normal ou ictérique", *C.R. Soc. Biol.*, **100**, 130.

Rutledge, E. K., and Neubuerger, K. T. (1942) "Icterus of the adult brain", *Amer. J. Path.*, **18**, 153.

Ryan, M. (1835). "Lectures on the physical education and diseases of infants from birth to puberty. No. 36", *London Med Surg. J.*, 7, 517.

Saint-Martin, A. (1948). "L'ictère hémoly[illegible]", *Rev. Méd.* [illegible]

Saint-Mart[illegible], *Rev. Méd* [illegible]

Saison R[illegible], 65, 71.

Sa[illegible] opsonique des anticorps anti-A [illegible] globules rouges homologues", *Rev. Hémat*, 11, 79.

Salmonsen, L. (1931) "Über [illegible] Erythro-Leukoblastose", [illegible]

[illegible], *Rev. méd. vét.*, 10, 468.

[illegible] des muletons en Poitou", [illegible]

Sausseau, L. (1925). "L'Ane, les chevaux mulassiers et la mule de Poitou" Paris, p 291.

Sborov, V. M, Jay, A. R., and Watson, C. J (1949) "The effect of aureomycin on urobilinogen formation and the fecal flora", *J Lab clin. Med*, 34, 1743

Schmorl, G (1903) "Zur Kenntnis des Ikterus neonatorum, insbesondere der dabei auftretenden Gehirnveranderungen", *Verhandl d. Deut. Path Gesell*, 6, 109 (Paper read in 1903; proceedings published in 1904.)

Schneider, L, and Szathmary, J (1938) "Uber die immunität der neugeborenen säugetiere", *Z ImmunForsch*, 94, 458, 465 and 95, 465

Scholten, R, and Veit, J. (1902) "Weitere Untersuchungen über Zottendeportation und ihre Folgen", *Zentralb f Gynäk*, 26, 169

Schridde, H (1910) "Die angeborene allgemeine Wassersucht", *Munch med Wschr*, 57, 397

Schulman, I, Smith, C. H, and Stern, G S (1954) "Studies on the anemia of prematurity", *Amer J Dis Child*, 88, 567.

Schultze, G (1676) "De Manna Abortus Causa", obs 241 on p 353 in "Miscellanea curiosa medico-physica Academiae naturae curiosorum sive Ephemeridum medico-physicarum Germanicarum", Annus VI and VII Frankfurt and Leipzig.

Schurig, M (1732) "Embryologia historico-medica" Dresden and Leipzig

Seifart, O (1899) Gerhardt's "Lehrbuch der Kinderkrankheiten" Tubingen

Sennert, D. (1633). "De infantum curatione tractatus", Pars II, Cap. XXII, p. 759.

Sherlock, S. (1955). "Diseases of the Liver and Biliary System." Blackwell, Oxford, p. 450.

Shumway, C. N., Miller, G., and Young, L. E. (1955). "Hemolytic disease of the newborn due to anti-A and anti-B", *Pediatrics, Springfield*, **15**, 54.

Simpson, J. Y. (1847). "Cases of fatal haemorrhage from the umbilical vessels in infants", *Month. J. Med. Sci. (Edinburgh)*, **8** (N.S. 2), 70.

Smith, C. A. (1951). "The Physiology of the Newborn Infant." Second edition. Oxford.

Smith, C. H. (1928). "Icterus neonatorum; its relation to compatibility of blood groups between mother and new-born", *Amer. J. Dis Child.*, **36**, 70

Smith, G. F. (1902). "A series of cases of jaundice in the foetus", *Lancet*, **ii**, 152.

Smith, P. (1875). "Case of abortion at six and a half months, with general dropsy of the foetus", *Transactions of the London Obstetrical Society*, **17**, 303.

Smith, S (1855) "Remarks on haemorrhage from the umbilicus of infants; with a table of 78 cases", *New York Jour. Med*, N.S., **15**, 73

Smith, E. L, and Holm, A. (1948) "The transfer of immunity to the new-born calf from colostrum", *J. biol. Chem*, **175**, 349.

Smith, T., and Little, R B. (1922) "Cow serum as a substitute for colostrum in new-born calves", *J exp. Med*, **36**, 453

Stemfel, R, and Zetterstrom, R. (1955) "Concentration of bilirubin in the cerebrospinal fluid in hemolytic disease of the newborn", *Pediatrics, Springfield*, **16**, 184

Stern, L., and Peyrot, R (1927). "Le fonctionnement de la barrière hémato-encéphalique aux divers stades de developpement chez les diverses espèces animales", *C R Soc. Biol*, **96**, 1124

Stetson, R E. (1933). "Causes and prevention of post-transfusion reactions", *Surg Clin N. America*, **13**, 319

Stratton, F (1953 *a*) "Detection of weak Rh antibodies in maternal antenatal sera. The value of enzyme-treated test cells", *Lancet*, **i**, 1169.

Stratton, F (1953 *b*) "A slide test for the detection of Rh-antibodies using papain-treated red cells", *Vox Sang*, **3**, 43

Swisher, S M, and Young, L. E. (1954). "Studies of the mechanism of erythrocyte destruction initiated by antibodies", *Trans. Ass. Amer. Phycns.*, **67**, 124.

Sylvius, F. D. (1679) "Opera Medica." Praxeos Medicae Appendix Tract. I, cap. 1, p. 589

[illegible]

Acta vet. hung, 4, 429

Szent-Iványi, T., and Szabó, S. (1952). *Mag. Allatorv. Lap*, p. 331.

Texier (1807). Mémoires de la Soc. d'Agric. des Deux-Sèvres.

Thomas, W. (1891). "Icterus neonatorum occurring in five successive births", *New Zeal. Med. Jour*, 4, 161

[illegible]

Ann Inst Past, 10, 63

van Bolhuis, J. H. (1948) "Placenta en Rhesusantagonisme" Leiden See also (1950) "Serological aspects of the placenta and other organs in the hemolytic diseases of the newborn caused by rhesus antagonism", *Maandschr Kindergeneesk.*, **18**, 106

van Loghem, J J, Kresner, M, and Verheul, C. (1950). "Serological investigations of the anti-human globulin serum (serum of Coombs) with special reference to the pathogenesis of erythroblastosis foetalis", *Maandschr Kindergeneesk*, **18**, 115.

van Loghem, J. J., Kresner, M., Coombs, R. R. A., and Roberts, G. Fulton (1950). "Observations on a prozone phenomenon encountered in using the anti-globulin sensitisation test", *Lancet*, ii, 729.
van Swieten, G. (1755). "Commentaries upon Boerhaave's Aphorisms concerning the Knowledge and Care of Diseases"
Vaughan, V. C., III, Allen, F. H., and Diamond, L. K. (1950) "Erythroblastosis fetalis; IV. Further observations on kernicterus", *Pediatrics, Springfield*, 6, 706.
Vaughan, V.C., III, Sotos, J. (1955). "Some serological observations on hemolytic disease of the newborn due to A or B incompatibility", *Amer. J. Dis. Child.*, 90, 531.
Vaughan, J. H., and Waller, M. V. (1957) "Immunologic features of erythrocyte sensitization. II. The nature of blood group antibodies", *Blood*, 12, 29.
Veall, N., and Mollison, P. L. (1950). "The rate of red cell exchange in replacement transfusion", *Lancet*, ii, 792.
Veit, J. (1900). "Untersuchungen über den osmotischen Druck zwischen Mütter und Kind", *Z. Geburtsh. Gynak.*, 42, 316.
Veit, J. (1904). "Verschleppung von Zotten und ihre Folgen", *Zentralb. f. Gynäk.*, 28, 1.
Villa Roya (1774) Cited by Bouley and Reynal, 1874.
Vogel, F. S. (1953) "Studies on the pathogenesis of kernicterus", *J exp Med*, 98, 509.
von Gierke, E. (1921) "Kernikterus und Erythroblastose", *Verhandl. d Deut Path. Gesell.*, 18, 322
Walker, W., and Murray, S. (1954). "The management of haemolytic disease of the newborn", *Brit. Med. J.*, ii, 126.
Walker, W., Murray, S, and Russell, J K (1957). "Induction of labour to prevent recurrent stillbirth due to haemolytic disease", *Lancet*, i, 348 see also Correspondence, *Brit. med. J.* (1957), i, 881
Walker, W, and Neligan, G A (1955) "Exchange transfusion in haemolytic disease of the newborn", *Brit Med J*, i, 681.
Wasastjerna, C. (1953) "Immunohaemolytic mechanisms in vivo", *Blood*, 8, 1042
Waters, W. J., and Bowen, W R (1955) "Bilirubin encephalopathy studies related to cellular respiration", *Amer J Dis Child*, 90, 603.
Waters, W J, Richert, D. A, and Rawson, H H (1954). "Bilirubin encephalopathy", *Pediatrics, Springfield*, 13, 319
Watson, H B., Crosse, V M, and Hatchuel, W. L. F. (1954). "Premature delivery in haemolytic disease of the newborn", *Brit. med. J*, i, 679.

Weichardt, W. (1901). "Moderne Immunitätslehre", *Münch. Med. Woch.*, 48, 2095.

West, C. (1859) "Lectures on the Diseases of Infancy and Childhood." Fourth edition. London, p 569.

Wiener, A. S. (1939). "Blood Groups and Blood Transfusion." [illegible]

Wiener, A. S. (195[illegible] [illegible]

W[illegible] 213

Wiener, A. S. [illegible] (1951). "Serum gamma [illegible]

W [illegible]

INDEX

Winternitz, M. (1926). "Urobilin beim Neugeborenen Zur Frage der Urobilinentstehung", *Klin. Woch*, 5, 988.

Witebsky, E. (1954). "The use of A and B substances", *Amer. J. clin. Path.*, 24, 321.

Witebsky, E., and Engasser, L. M. (1949). "Blood groups and subgroups of the newborn. I. The A factor of the newborn", *J. Immunol.*, 61, 171.

Witebsky, E., Rubin, M. L., and Blum, L. (1947). "Studies in erythroblastosis fetalis. I. Activation of the incomplete Rh antibody by the blood-serum of full-term and premature newborn infants", *J. Lab. clin. Med.*, 32, 1330.

With, T. K. (1945) "On the occurrence in human serum of yellow substances different from bilirubin and carotenoids", *Act. Med. Scand*, 122, 501.

Wootton, I. D. P. (1950). "Production of Coombs's serum", *Nature (Lond.)*, 165, 730.

Wootton, I. D. P. (1951). "Factors affecting the speed of agglutination in the Coombs' test", *J. clin. Path.*, 4, 296.

Wrisberg, H. A. (1764) "Descripto anatomica embryonis", Obs I Gottingen

Young, L. E., Ervin, D. M, and Yuile, C. L. (1949). "Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma I. Serologic and hematologic aspects", *Blood*, 4, 1218.

Young, L. E., Ervin, D. M., Christian, R. M., and Davis, R. W. (1949). "Hemolytic disease in newborn dogs following isoimmunization of the dam by transfusion", *Science*, 109, 630.

Young, L. E., Christian, R. M, Ervin, D. M., Davis, R. W, O'Brien, W. A., Swisher [illegible] Yuile, C. L. (1951). "Hem[illegible], *Blood*, 6, 291

Zach[illegible] between blood of the san[illegible] irregular agglutinin and of [illegible] described *receptor*", *Hopsitals tid.*, 78, 225.

Zetterstrom, R, Stempfel, R., and Escardó, F. E (1956) "Methemalbuminemia in the neonatal period with special reference to hemolytic disease of the newborn", *Acta Pediat., Stockh*, 45, 241

Zuelzer, W. W., and Kaplan, E. (1954). "ABO heterospecific pregnancy and hemolytic disease" (four papers), *Amer. J Dis Child*, 88, 158, 179, 307, 319

Zuelzer, W. W., and Mudgett, R T. (1950). "Kernicterus; etiologic study based on an analysis of fifty-five cases", *Pediatrics, Springfield*, 6, 452.